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# SOIL SCIENCE

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# STATIC FRICTION MEASUREMENTS IN THE STUDY OF SOIL MOISTURE RELATIONSHIPS<sup>1</sup>

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The relationship between soil particles and the water films that surround them has always interested soil scientists. The layers of water that surround the individual particles of the soil are obviously bounded by two kinds of interfaces: the inner one, a phase boundary between a solid and a liquid; and the outer one, a phase boundary between a liquid and a gas. Although there is some information available concerning the activity of surface tension forces at the air-water interfaces in soils, little is known about the active forces at the water-soil interface, which must be of molecular character and which are probably of relatively great magnitude. The distance through which such forces act at the latter phase boundary and the possibility of discontinuity at certain points in the moisture range are of especial interest.

## REVIEW OF LITERATURE

The dilatometer studies of Bouyoucos (3) seemed to indicate that there is an abrupt change in the characteristics of water a short distance away from the solid-liquid interface in a soil-water system. Below a certain point in the temperature scale it was found impossible to freeze any more water in wet soils. The amount of this unfree water varied with the amount of internal surface, clays holding much more water against the freezing processes than did sandy soils. Bouyoucos has suggested that this unfreezable water is no longer in the liquid state but exists as water of hydration or water in solid solution. This is not necessarily true, since the formation of ice crystals merely requires a rearrangement of water molecules in a definite crystal lattice pattern, a process which may be easy in the absence of restraining forces but probably difficult if the water molecules are already oriented and strongly held at a solid-liquid interface. When the force of attraction and orientation is greater than the force of crystallization, it is probable that no more ice crystals will form.

Vapor pressure measurements within a soil made by Edleson (5) and others seem to indicate a discontinuity and an abrupt change in state a short distance from the interface because vapor pressure is at maximum value over a long

<sup>1</sup> Published as Scientific Paper No. 308, College of Agriculture and Experiment Station, State College of Washington.

range in soil moisture and then suddenly drops to about zero near the wilting point.

Atterberg's studies (1) indicated rather abrupt changes in the consistency of clays when the moisture content was varied. Abrupt color changes often occur as clays dry out.

During the war, Hardy and Hardy (7) made extensive studies of the ability of different liquids to overcome friction. They used several organic liquids, including acids, and water and studied their effect on the friction between glass surfaces. They found that water was not a good lubricant even when flooded on such surfaces. The more strongly polar compounds were most active in overcoming friction. Hardy used measurements of static friction rather than measurements of moving friction because of the simplicity resulting from the important fact that the coefficient of static friction is entirely independent of the area in contact. This seemed to commend the measurement of static friction in connection with soil investigations where exact surface areas are usually difficult to ascertain.

Soon after Hardy's work appeared, Crowther and Haines (4), of the Rothamsted Experimental Station, reported studies in which they placed a negative charge on plow mold boards, which resulted in a movement of water to the metal-soil junction and reduced the drawbar pull through reduced friction with the soil. Laboratory studies of sliding friction between metal surfaces and moist soils were also made, which agreed with the field experiments. In these studies it was found that the higher the moisture content of the soil, the greater was the reduction in kinetic friction due to the electric charge.

Baver (2) reported work with the coefficient of kinetic friction at various moisture contents and showed the relationship of these values to the Atterberg constants. He concluded that the upper plastic limit of Atterberg corresponds to the point of maximum adhesion. Atterberg, however, determined the point of maximum adhesion and found that it occurs in the range of moisture between the upper and lower plastic limits, a point which he termed the "sticky limit."

The determination of coefficients of static friction at different moisture percentages appeared to be a unique means of studying the question of soil-water relationships, and, since there is obvious disagreement about the nature of the moisture films at the solid-liquid interface in moist soils, the experiments reported here were undertaken.

#### SOILS

Six clays were selected for study. They were obtained from widely separated points and were representative of at least three types of soil formation. In order to eliminate the effects of organic matter as much as possible, samples of deep horizons were used. The percentage of clay smaller than 0.002 mm. in diameter was determined by the Robinson pipette method. Atterberg constants were found according to the directions of this investigator. The

colors were checked against Ridgeway's color standards, table 1 shows the results.

A chemical analysis was made of sodium carbonate fusions according to the usual methods, and sodium and potassium were determined directly from J. Lawrence Smith fusions, using the zinc-uranyl acetate method and the cobalt-nitrite method respectively. The results, together with calculations of the silica sesquioxide ratio, are given in table 2.

TABLE 1  
*Some physical characteristics of the clays used*

SOIL	LOCALITY	COLOR*	CLAY CON- TENT	ATTERBERG CONSTANTS		
				Rolling limit	Flow limit	Plasti- city number
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Fortuna.....	Honduras, C. A.	Ferruginous	53.0	35.9	53.4	17.5
Spur Tree.....	Jamaica, B. W. I.	Morocco red	†	39.1	43.1	4.0
Hagerstown.....	Hagerstown, Pa.	Cinnamon rufous	56.0	28.7	54.8	26.1
Cecil.....	Rockville, Md.	Apricot orange	48.0	36.2	47.7	11.5
Napance.....	Ypsilanti, Mich.	Pale smoke gray	57.0	20.7	36.4	15.7
Kalispell.....	Cusick, Wash.	Gull gray	66.0	38.2	67.1	28.9

\* From Ridgeway.

† Dispersion not successful.

TABLE 2  
*Chemical analysis of the clays used*

SOIL	RATIO SiO <sub>2</sub> R <sub>2</sub> O <sub>3</sub>	PERCENTAGE						
		SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	Ca	Mg	K	Na
Fortuna.....	2.50	51.94	31.95	5.20	0.40	1.08	0.43	0.34
Spur Tree.....	0.10	3.98	61.10	10.10	0.90	0.62	0.18	0.44
Hagerstown.....	3.20	57.93	27.40	5.20	1.01	1.74	0.97	0.50
Cecil.....	3.07	57.19	27.50	6.40	0.50	1.45	2.30	0.51
Napance.....	5.25	44.79	12.70	2.80	9.70	3.24	2.27	0.79
Kalispell.....	4.90	65.05	20.03	3.40	1.30	2.46	2.04	1.06

#### MEASUREMENTS OF STATIC FRICTION

The method of Hardy and Hardy was employed except that shallow pans filled with moist soil were substituted for the lower glass plate. A new watch glass about 6 cm. in diameter and perfectly smooth on the bottom was fitted with a hook on the upper side, extending to the edge of the glass. One end of a piece of silk thread was attached to the hook, and the other end was fastened to a balance pan. A beaker support was attached to a ring stand with a short upright, and opposite to the beaker support was placed a rod having a metal

wheel at the end. The wheel was almost frictionless and was adjusted in such a manner that the silk thread extending over it to the watch glass when resting on the soil was parallel to the soil surface. Metal pans 8 cm. in diameter and 0.4 cm. deep were filled evenly with thoroughly wetted and mixed soil, and the surface was smoothed with a spatula. The pans were placed on the beaker support, which was perfectly level when measurements were being made.

In making a measurement the pan of soil was placed in its support after the surface had been carefully smoothed, and the watch glass loaded with 30–40 gm. of metal shot was carefully placed on the soil. The silk thread was laid over the metal wheel so that the balance pan, hanging over the edge of the table, was above a support placed about 1 inch below the pan. Shot was then slowly added to the balance pan until the watch glass just started to move. Since static friction is always greater than moving friction, the glass rapidly moved over the soil once it had started. The object of the experiment was to add only enough shot to start the glass moving. A fraction of a gram less than this weight is the true value to be sought. This is spoken of as the "threshold value" and represents the force necessary just to overcome the force of static friction. The test being completed, both watch glass with shot and balance pan with shot were weighed and the data recorded. The soil was again smoothed, the watch glass with a larger load of shot replaced in position, and the process repeated. A third trial with a larger load of shot, preferably about 100 gm., was made. When this reading was obtained, duplicate samples of the soil near the center of the plate were taken out and placed in weighed dishes with tight covers for moisture determinations. These samples, after being weighed, were dried in an oven at 105°C. and then reweighed. Moisture percentages at the time of the friction determinations were then calculated, the percentage being based on the weight of oven-dried soil.

The coefficient of static friction ( $U$ ) is calculated from the relationship  $U = \frac{P}{L}$ , where  $P$  is the weight required just to start the load ( $L$ ) moving on a plane surface. The value of  $U$  is always less than unity (8). Because the amount of surface area in contact has no effect on this coefficient, the ratio  $\frac{P}{L}$  is a linear function for a given pair of surfaces, and it is possible to standardize the load at any convenient point along the curve. For these experiments a standard load of 100 gm. was selected. The nature of the linear curves obtained with a sample of Hagerstown clay is shown in figure 1. The corresponding moisture content is shown with each set of determinations. It will be noticed that the slope of the curve is not always the same but that the experimental points are always in linear relation. The coefficient of static friction with any load can be read from these curves.

Storage of the thin plates of soil in an enclosed desiccator of large size, but

without any drying substance in it, resulted in a gradual loss of moisture from the soil; but capillarity maintained a uniform moisture content from top to bottom of the plate. After suitable periods of time, when an appreciable loss in moisture had occurred, new measurements were made. By starting the experiment with very moist samples and allowing the soil to lose water slowly,

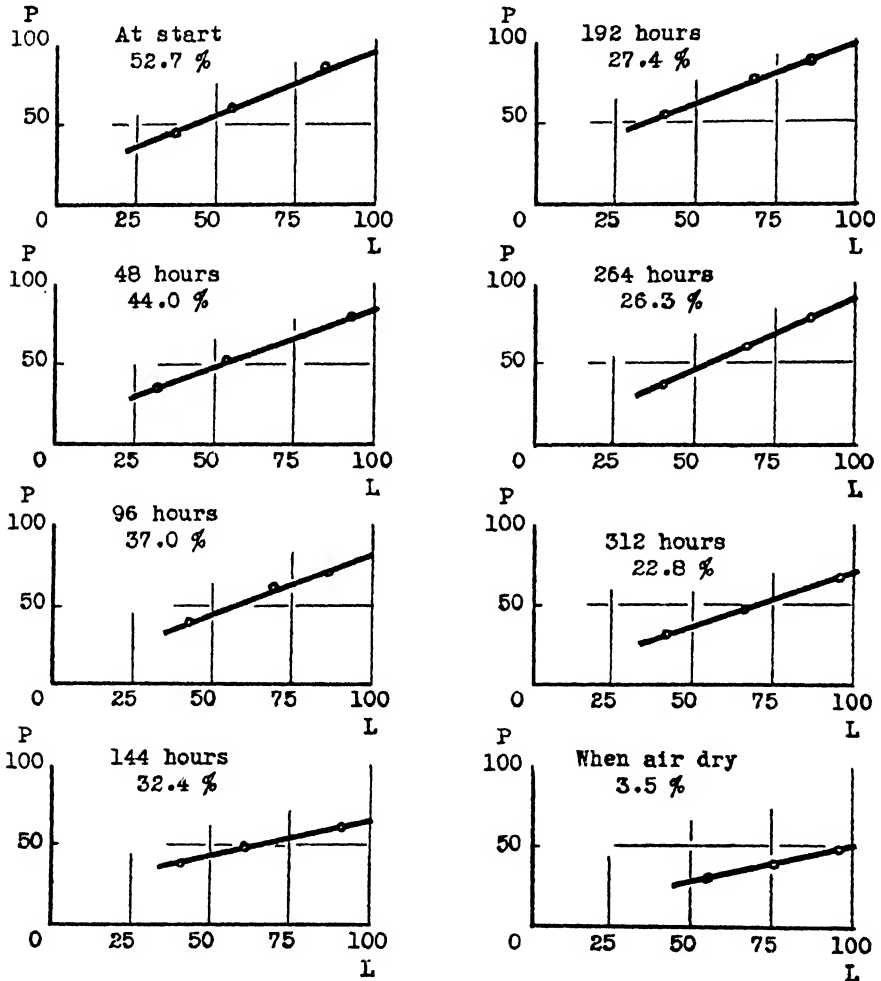


FIG. 1. THE LINEAR RELATION BETWEEN THE PULL ( $P$ ) AND THE LOAD ( $L$ ) IN STATIC FRICTION MEASUREMENTS AT DIFFERENT MOISTURE PERCENTAGES IN HAGERSTOWN CLAY

a series of measurements along the entire moisture range was made on the same plate of soil. As samples are removed, the soil may be worked to one side of the plate for smoothing and testing. The time required for a complete run from very moist soil to the air-dry condition varied with different soils. That for Hagerstown clay is indicated in figure 1.



## EXPERIMENTAL RESULTS

The variations in the coefficient of static friction with changing moisture contents in six clays are shown by means of graphs (fig. 2, 3, 4). The coefficient of static friction is shown as the dependent variable, and the moisture content as the independent variable. The Honduras and Jamaica soils are shown together in figure 2, since both are lateritic soils and the latter may be a true laterite. Figure 3 shows the results with the Cecil and Hagerstown clays, both of which are residual soils. Results with two glacial soils as represented by the Napanee and Kalispell clays are shown in figure 4.

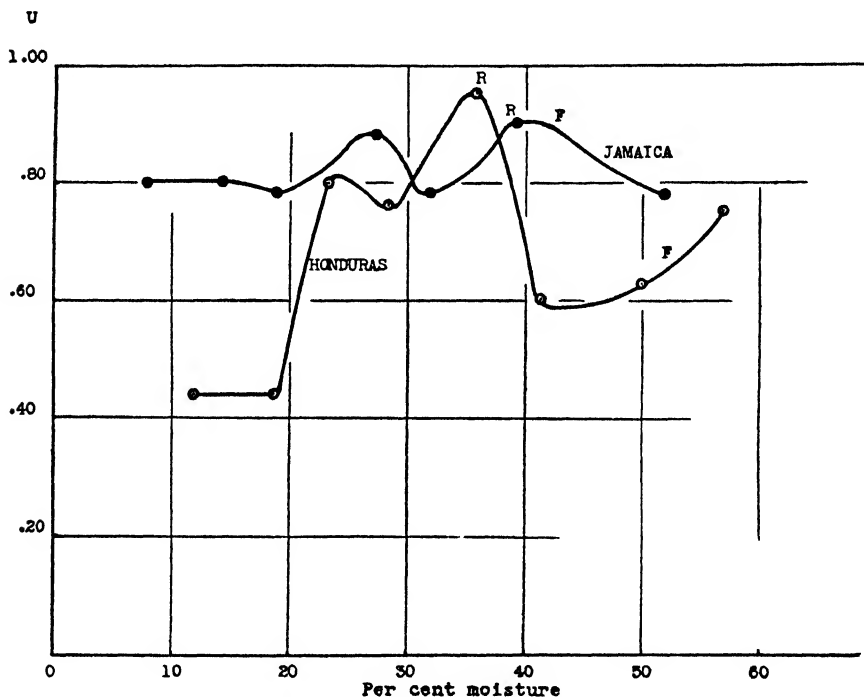


FIG. 2. CHANGE IN THE COEFFICIENT OF STATIC FRICTION WITH INCREASING MOISTURE CONTENT OF THE HONDURAS AND JAMAICA CLAYS AND THE RELATIONSHIP OF THE CURVES TO THE ATTERBERG CONSTANTS  
*R* and *F* represent rolling and flow limits respectively

It will be observed that in all cases the coefficient of static friction increases to a maximum value after passing a moisture content that closely corresponds to the wilting point as determined by separate studies according to the Bouyoucos method, drops to a low point, and then gradually rises again. In some cases a sub-maximum value was indicated. The rolling limit was found to correspond to the point of maximum friction in every case. The flowing limit is not definitely associated with any particular point on the friction curve but tends to fall near the beginning of the second rise in the curve. It should be

noted that, after the flowing point is passed, the soil particles are free to move over one another and the loaded watch glass sinks into the moist clay and meets with a mechanical resistance similar to that of plowing. The wetter the soil becomes, the deeper the glass sinks and the greater is the resistance to movement. Consequently, a rise in the curve after passing the flowing limit is not a true measure of friction at these moisture percentages. It is more than likely that the friction constantly approaches a very low value as the soil becomes more and more liquid, since the friction of glass on a pure liquid surface is comparatively low.

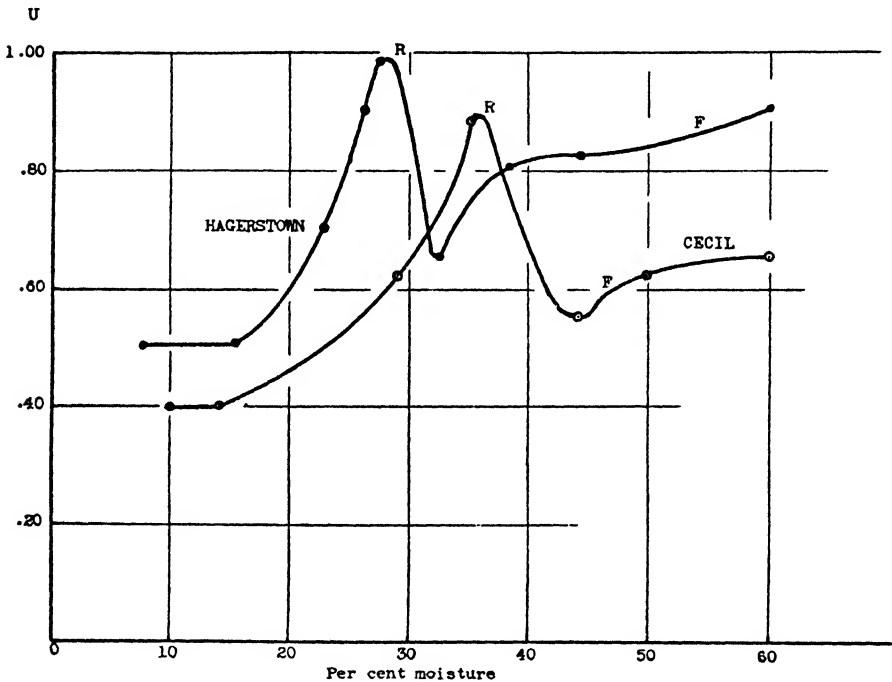


FIG. 3. CHANGE IN THE COEFFICIENT OF STATIC FRICTION WITH INCREASING MOISTURE CONTENT OF THE HAGERSTOWN AND CECIL CLAYS AND THE RELATIONSHIP OF THE CURVES TO THE ATTERBERG CONSTANTS  
*R* and *F* represent rolling and flow limits respectively

The rise in the coefficient of static friction as the soil is wetted above the wilting point is obviously associated with the development of a strong attractive force between the glass and the soil particles through the agency of water. The water films which form around the soil particles at these moisture percentages must be tenacious. The rise here cannot be attributed to increased surface energy resulting from increasing the surface of the air-water interfaces which exist as menisci between the wet soil and the watch glass because increasing the contact surface has no influence on the coefficient of static friction. The explanation must be sought for at the soil-water interface.

At the lower moisture contents the film was probably discontinuous and the water molecules were so scattered that only a few contacts were made with the glass surface. It is possible also that much of the moisture held at these percentages was found in small capillaries or even within the crystal lattice and was not connected with the interface. As the soil is wetted, more truly interfacial water appears, more contacts are made, and friction increases to a certain point, after which a drop occurs.

U

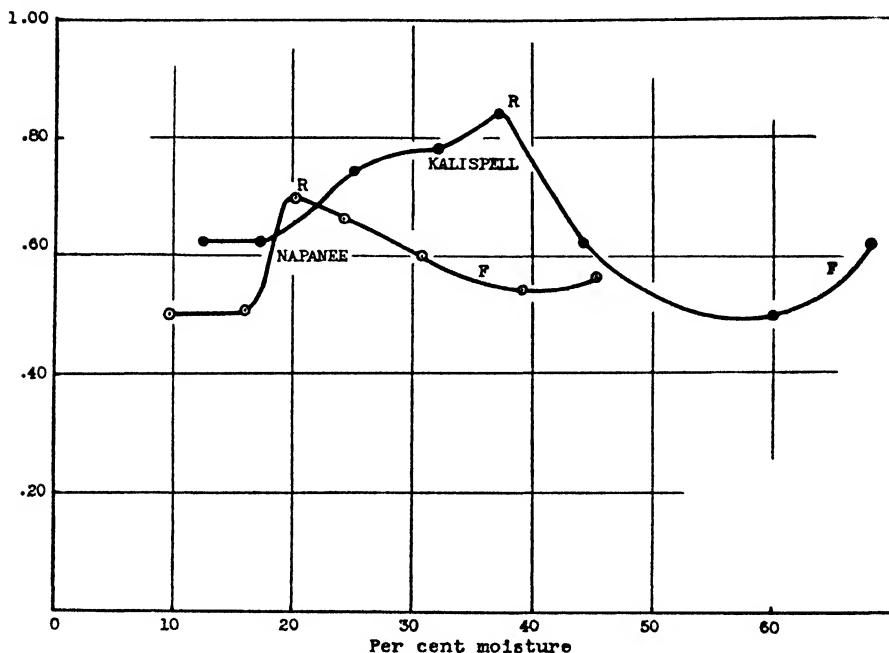


FIG. 4. CHANGE IN THE COEFFICIENT OF STATIC FRICTION WITH INCREASING MOISTURE CONTENT OF THE NAPANEE AND KALISPELL CLAYS AND THE RELATIONSHIP OF THE CURVES TO THE ATTERBERG CONSTANTS  
R and F represent rolling and flow limits respectively

At this point there must be some abrupt change in the character of the moisture films. One explanation is to be found in the probable complete orientation of water molecules at the solid-liquid interface at low moisture contents, which keeps them in a semi-rigid condition. Because they attach themselves to the glass as well as to the particle surfaces, it is difficult to overcome the adhesive force as long as complete orientation occurs. A progressively more dense layer of such oriented water molecules is probably built up as the soil is wetted more and more. The process may be thought of as the addition of more and more connecting links between the soil particles and the glass surface, each link being more or less rigid and adhering to soil and glass surfaces at

opposite ends. Eventually, at the point when the maximum friction occurs, the molecules of further additions of water are probably no longer oriented and they are free to adjust themselves when a tangential force is applied. The friction is then reduced. When further additions of water are made, increased freedom of molecular adjustment is attained within the water film, and the glass moves progressively more easily as it approaches the moisture content at the flowing limit.

In this connection it is of interest to consider more carefully the discussions of Hardy and Hardy with respect to static friction between glass surfaces. They say:

The surface film (of fluid or solid) must therefore have a characteristic molecular architecture and the (resulting) condition of minimal potential involves two terms, one relating to the variation of density, the other to the orientation of the fields of force of the molecules. Any polarization of molecules at the surface must introduce a factor in the resistance to (tangential) slip which is absent from any resistance there may be to a displacement along the normal, for the former includes the resistance which the molecules may offer to disturbance of their orientation.

Surfaces whose properties depend upon the interaction of two kinds of states of matter have been called "composite surface."

Two consequences follow—the film resists tangential displacement and therefore has tenacity, and since the potential energy of the molecules composing it is a function of their position, the film is not completely fluid even though it be formed of material which is a fluid when in mass at the same temperature and pressure. Owing to this defect in fluidity composite surfaces are capable of seizing and the static friction of such surfaces is, in our opinion, due to this fact. By seizing is meant the capacity for offering resistance to slip when both faces are at rest.

These investigators found that water in thin films of the order of  $1\mu$  was not a lubricant, though in thick films it appeared to be one. The explanation for the rise in static friction in soils, as previously suggested, probably lies in this tendency to seize, and the indications are that up to the point of maximum static friction in soils only oriented water molecules are present, increasing in density per unit area as the moisture content increases. When the friction begins to decrease, the formation of a thicker film in which the molecules are free to move is suggested. If the water molecules in the moisture films were passive, then the increase in the number of contacts between the glass and the soil as the molecular density per unit area of film increased would be ineffective insofar as static friction is concerned, since this would simply amount to an increase in the contact area of inactive surfaces, which is known to bear no influence. The result of a passive condition of the water molecules is shown in the horizontal portion of the friction curves, extending from the air-dry condition to the region of the wilting point.

From these considerations it appears that soil moisture to the lower plastic limit exists as a rigid film of the order of  $1\mu$  in thickness. Hardy (6) believes that there may be more than one layer of oriented molecules in a composite surface such as this one. That this may be the case in soils as the rolling limit

is approached seems reasonable because there is probably not enough internal surface to hold the percentage of water present in the soil at this point and have a water layer but one molecule thick. After the point of maximum friction is passed, free moving molecules of water are present, and the forces of fluid surface tension come into play. This is the beginning of the plastic state. At the upper plastic limit the amount of free water is sufficient to cause plastic flow if a liquid-solid phase replaces the solid-liquid phase which characterized the plastic state. Consequently, there are two distinct points of discontinuity indicated in connection with the soil-water system, and the location of these points on the moisture scale corresponds generally with those obtained by Atterberg for his rolling limit and flow limit.

There is some relationship between the magnitude of the coefficient of static friction and the plasticity number in the six clays, but no numerical relationship is suggested, probably because of the wide differences in both colloidal content and chemical composition. The most striking relationship occurs in the case of the glacial clays. The maximum coefficient of friction for the Kallspeck clay is 0.84 and that for the Napanee is 0.60; the respective plasticity numbers are 28.9 and 15.7. The Jamaica sample, with a low plasticity number, exhibits only a small rise and fall in friction values, whereas the clays, with high plasticity numbers, exhibit wide variations in friction coefficients at different moisture contents.

#### DISCUSSION

It is significant that the plastic properties of clays are associated with a certain range in moisture content. The manifestation of plasticity must be intimately connected with the soil-water relationship.

Plastic properties and friction coefficients are no doubt accounted for by the fundamental concept of simple molecular attraction. From a consideration of the work of Hardy and others and orientation of molecules at phase boundaries and from the nature of the changes in static friction at different percentages of soil moisture as shown here the soil moisture relationships appear to be as follows:

In air-dry soil the soil moisture exists in capillaries in the crystal lattice or, at best, in disconnected surface patches with the water molecules oriented and immobile except when heated.

At the wilting point a continuous interfacial film is formed by oriented water molecules, which cover the particle surfaces but are still held strongly by interfacial molecular forces.

At the rolling limit or lower plastic limit the composite film with its oriented water molecules, which are possessed of considerable tenacity, reaches its maximum thickness. This is the point where some freedom of movement among the water molecules is just beginning and a plastic condition appears.

At the upper plastic limit or at the flowing limit a reversal of phases occurs corresponding to a change from a liquid in a solid to a solid in a liquid system.

The answer to the question of the lubricating qualities of water depends on the relationship between the water and the solid interface to which it is at-

tached. When strongly held at the interface through orientation of its molecules, water is not a lubricant but, when its molecules are free to move, it may act like a lubricant to a limited degree.

Varying degrees of plasticity seem to be associated with varying degrees of freedom of movement of water molecules at the solid-liquid interfaces. Surface tension effects at liquid air interfaces within a soil are not enough to account for plasticity or tenacity because in puddled plastic clays such interfaces may not exist. It is likely that the interfacial forces at the solid-liquid phase boundary are of greatest importance in accounting for many of the physical characteristics of clays.

#### SUMMARY

Static friction measurements having been successfully used in the study of liquid films between glass surfaces, the method was adopted for the study of water films on the surface of clay particles. Six clays were studied over a wide range of moisture, and certain relationships between maximum and minimum points on the curves for static friction and the Atterberg constants were found. The results indicate that the interfacial forces at the soil-water phase boundary are extremely important in the development of many physical properties of soils.

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# THE EFFECT OF REPLACEABLE BASES ON THE PHYSICAL PROPERTIES OF SOILS WITH SPECIAL REFERENCE TO THE EFFECT OF REPLACEABLE CALCIUM AND SODIUM ON INDEX OF FRIABILITY<sup>1</sup>

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Investigations have shown that replaceable calcium and sodium play an important rôle in the reclamation and tillage of agricultural lands in the West. It is generally conceded that physical properties of the soil such as structure, dispersiveness, permeability, cohesive nature, plasticity, water-holding capacity, and friability are affected by the replaceable cations—sodium and calcium. The cation calcium has a desirable effect on the soil, whereas the undesirable effect of the cation sodium can be found in the heavy, cloddy, poorly-drained soils of the West. Most of the high-producing, friable soils of the West and Middlewest have a high percentage of replaceable calcium in the colloidal fraction of the soil. Gedroiz (4) states: "When the soil-absorbing complex is saturated with sodium in a moistened condition it will be a viscous and sticky mass which becomes, on drying, so hard and compact that it is broken into pieces with difficulty and only by the use of high pressures." He is of the opinion, however, that as long as the total replaceable bases in the absorbing complex contain more than 80 per cent calcium, the soil will have maximum friability. The purpose of this experiment was to attempt to measure this change in friability upon soils treated chemically in the laboratory.

Many ways have been suggested for measuring the friability of soils. Workers in the United States Bureau of Chemistry and Soils (2) attempted to measure it by the resistance of the soil to a sharp tool. English workers (5) tried the indirect method of investigating cohesion and plasticity. Middleton (9), in the United States Bureau of Chemistry and Soils, measured the binding power of soils and colloids by means of machines that measured the compressive strengths of briquettes. Stauffer (11) approached the problem from the angle of the tensile strength of the soil. Christensen (3), following the lead of Stauffer, developed a special friability test. He defines the index of friability as the ratio of the unit deformation at the yield point to the work of deformation

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at this point. It has the dimensions of the reciprocal of a force per unit area. Christensen's apparatus and method with modifications were used in this work.

The soil used in the present study was a heavy clay soil in poor physical condition collected from the Bell tract near Logan, Utah. The samples used were Nos. 1867 and 1872 used by Harris (6) for his studies on permeability, the former containing small amounts of calcium carbonate (and possibly magnesium carbonate), and the latter, only a trace of inorganic carbonates. No. 2784 (collected directly from the field by the authors) contained small amounts of inorganic carbonates and 0.66 per cent of soluble salts, as determined by the electric bridge method. A chemical analysis of the soluble salts showed chloride and bicarbonate to be the predominating anions and sodium the predominating cation. This sample was used for most of the experimental studies.

#### METHOD USED IN DETERMINING INDEX OF FRIABILITY AND RELATED PHYSICAL PROPERTIES

Index of friability and related physical properties were determined as follows: 210 gm. of air-dry soil were placed in a large casserole and stirred continuously while water was added slowly by means of a wash bottle. When the soil became wet enough to mold readily and yet was not sticky, it was taken into the hands, and the mixing of the soil and water was continued until the soil paste reached a uniform consistency. The wet soil was then molded into a long cylinder with a diameter slightly less than that of the mold. (A steel mold with a diameter of 2.7 cm. was used for these determinations. The length of the mold was adjusted to 3.37 cm. by means of a piston and threaded cap.) Portions of the soil cylinder were pressed firmly into the mold, smoothed off, removed, and weighed. The soil cylinders thus produced (usually five) were dried for 72 hours in a moist atmosphere and finally at a temperature of 110°C. for 24 hours. The cylinders were cooled in a desiccator, weighed, and the length and diameter of each measured. The compressive strength of each cylinder was determined in the testing machine by obtaining the weight necessary to crush the soil cylinder at the yield point. From data thus obtained, the following physical properties were calculated, using the units indicated: (a) shrinkage coefficient (volume of mold taken as standard), (b) moisture for molding ( $M$ ) [dry (110°C.) soil = 0.0 per cent], (c) apparent density of dry ( $\rho$ ) (110°C.) soil, and (d) index of friability ( $I$ ) i.e., reciprocal of compressive strength per unit area =  $1000 \frac{\text{cm.}^2}{\text{kgm.}}$ . Physical data shown in figures 1 and 2 and in tables 1, 2, and 3 represent an average for all the soil cylinders molded at one time from each respective 210-gm. sample.

#### FACTORS INFLUENCING THE INDEX

At the beginning of this work, difficulty was experienced in duplicating results of friability measurements. Variations in density of the soil cylinders

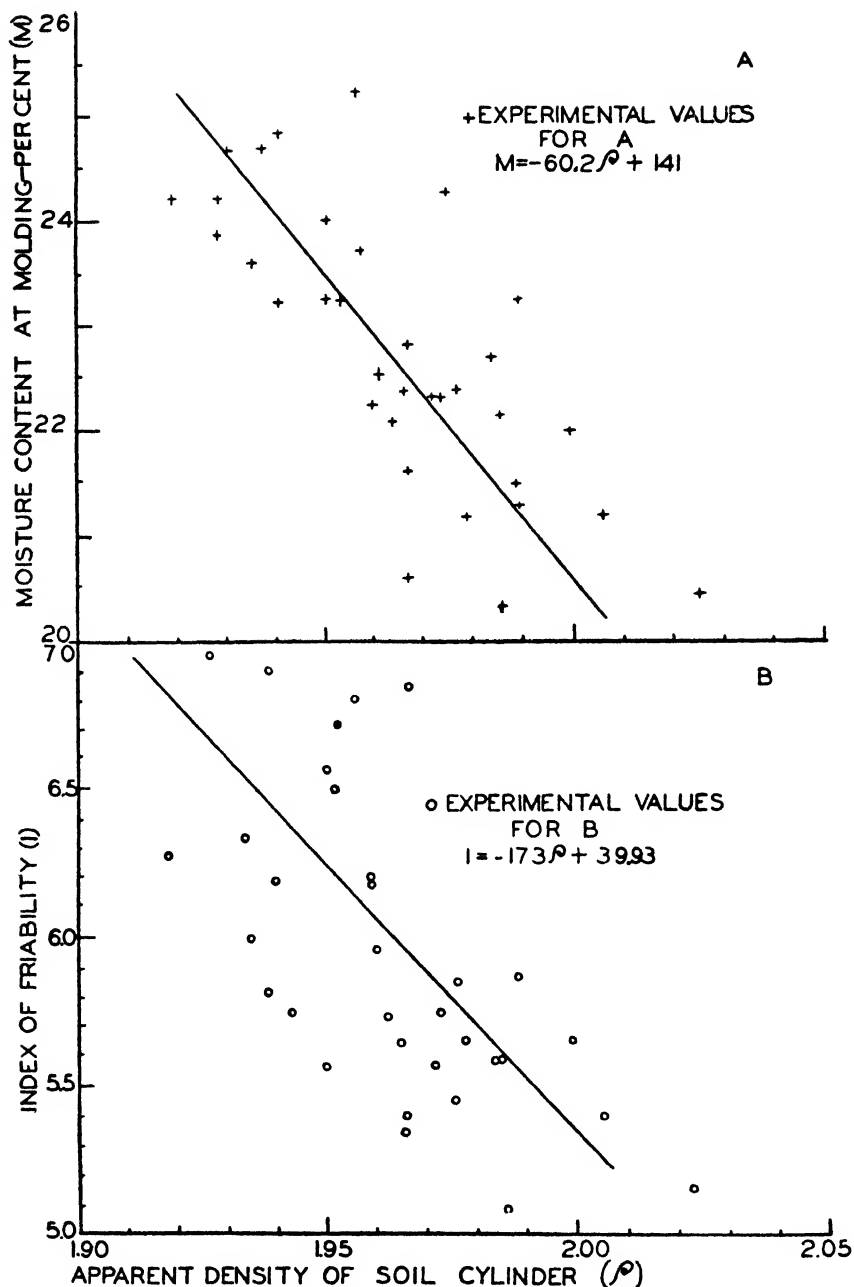


FIG. 1. (A) RELATIONSHIP BETWEEN MOISTURE CONTENT AT MOLDING AND APPARENT DENSITY OF SOILS. (B) RELATIONSHIP BETWEEN APPARENT DENSITY OF SOILS AND INDEX OF FRIABILITY

was suspected as being the principal cause. This variation was probably due to (a) the manner in which the soil is ground and mixed with water; (b) the amount of water used in molding; or (c) the amount of pressure used in packing the soil into the mold. A quantitative study using Soil No. 2784 was made for factor (b), keeping factors (a) and (c) constant. The statistical relationship of this moisture to apparent density is shown graphically in figure 1-A, and its relationship to index of friability, in figure 1-B. Assuming the relationships to be straight lines within the range of the experiment, the equation for the former is  $M = 60.2\rho + 141$ , and that for the latter  $I = 17.3\rho + 30.93 \pm 0.24$ , where  $M$  = moisture for molding,  $I$  = index of friability, and  $\rho$  = apparent density.

There was much less variation in the index of friability when the apparent density was above 1.97 than when below this value (fig. 1-B). This is brought out more forcibly by determination of the standard error of estimate. When the average index of friability for the five soil cylinders is used as the unit,

TABLE 1  
*Effect of washing soil on index of friability*  
Soil No. 2784

SUBSTITUTE NO.	BEFORE WASHING			AFTER WASHING		
	Moisture for molding	Apparent density	Index of friability	Moisture for molding	Apparent density	Index of friability
	<i>per cent</i>			<i>per cent</i>		
8	22.8	1.967	5.42	22.6	1.971	5.52
12	22.5	1.976	5.48	22.6	1.952	5.77
13	22.2	1.964	5.74	22.6	1.974	5.77
15	21.3	1.978	5.64	22.8	1.956	6.06

this value for the entire density range is  $\pm 0.24$ . If only cylinders with an average index lower than 1.97 are considered, this value is  $\pm 0.28$ , whereas, if only those with an index greater than this are considered, the value reduces to  $\pm 0.16$ . Therefore, in order to obtain the most reliable results for soils similar to Soil No. 2784, it is advisable to produce cylinders with an apparent density above 1.97. This may be done by using just enough water to mold the soil readily, which for this soil was approximately 22 per cent.

Another factor which influences the index is the rate of drying of the soil cylinders. When they were dried too fast, they cracked and the compressive strength of the cylinders was lowered.

All of these factors can be fairly well controlled by carefully following the same method of molding and drying and by using the same amount of water for wetting the soil. These results confirmed the finding of Middleton (9), working in the United States Bureau of Chemistry and Soils with briquettes of soil colloids.

Some samples of soils were washed free of soluble salts in Pasteur-Chamber-

land filters to determine whether their removal from the soil had any effect upon the index of friability. An examination of table 1 shows that washing had little effect. It seems probable that if soils containing large amounts of alkali were washed free from soluble salts, a difference in friability would be noted, as the soluble salts present in the soil solution may have a flocculating effect on the colloidal material or they may affect the replaceable base equilibrium in the soil.

#### CHEMICAL TREATMENT OF SOILS

According to the present-day theory, replaceable base reactions take place primarily in the colloidal fraction of the soil. The bases exist as portions of true chemical compounds and can be removed from the soil only when another base or hydrogen takes their place. Several methods have been used for replacing bases in soils. The one used in this work was as follows:

Two hundred and ten grams of soil were given a substitute number, divided into three 70-gm. portions, and each placed in a large Erlenmeyer flask, with 250 cc. of a normal chloride solution. The cation associated with the chloride in the treating solution was either a single base, such as sodium or calcium, or a mixture of two bases. The flasks were shaken several times a day, and the suspension was allowed to settle overnight. The following morning the clear solution was syphoned off, and 250 cc. more solution was added. This was repeated several times, usually for 8 or 10 days.

After the required number of applications, the soil was washed five times through a Pasteur-Chamberland filter in order to remove the soluble salts. Before each washing, the entire 70 gm. of soil was shaken with distilled water in a mechanical shaker. After washing, the three 70-gm. portions of soil were mixed and allowed to dry. The water required to remove the soil from the filter was evaporated at about 40°C., and the bulk of the soil was dried at room temperature. After drying, the soil was ground to pass a 1-mm. sieve and then thoroughly mixed. This entire procedure is spoken of as a "treatment."

#### ANALYSIS FOR REPLACEABLE BASES<sup>3</sup>

The method used to remove the replaceable bases from the soil for analysis was similar to the first part of the treatment as described. A 15-gm. portion of soil was treated with six applications of an aqueous solution of 0.1 *N* ammonium acetate, and a separate 15-gm. portion was treated with seven applications of 0.1 *N* potassium chloride in 50 per cent alcohol. All treatments were run in duplicate. The solutions syphoned from the six ammonium acetate applications were made up to a given volume, and aliquot portions were analyzed for sodium and potassium, respectively. Sodium was determined

<sup>3</sup> The method used to determine calcium and magnesium in calcareous soils was one developed by Joel E. Fletcher in this laboratory (unpublished).

by the zinc uranyl acetate method, and potassium, by the perchloric acid method.

Most of the soils studied contained some calcium carbonate (and possibly magnesium carbonate); therefore, in order to make the determinations of replaceable calcium and magnesium more reliable, it was necessary to reduce the solubility of their carbonates to a minimum and make a correction for the solubility factor. The method used was based on the assumption that the solubility remained constant throughout the treatment.

Solutions syphoned from the first five applications of 0.1 *N* potassium chloride solution were mixed and made up to a given volume, and an aliquot portion was analyzed for calcium and magnesium. Solutions from the sixth and seventh applications were mixed and analyzed for soluble calcium and magnesium. Replaceable calcium and magnesium were calculated by subtracting 5/2 of the respective amounts found in the latter determination from the amounts found in the former.

#### GENERAL EXPERIMENTAL PROCEDURE

After the method for determining the index of friability had been well standardized, the following methods of procedure were used to determine the relationship between replaceable bases and various physical properties. The soil was ground to pass through a 1-mm. sieve before each determination.

The index of friability and related physical properties were determined for each soil sample, as described, before any treatments were made and again after the soil had been given the chemical treatments indicated in the following. The data are recorded in tables 2 and 3. Only the average value for the untreated soil is given.

Soil samples were treated with eight or ten applications of the respective single chlorides, sodium, calcium, and magnesium, and with various mixtures of sodium and calcium chlorides, as indicated in table 2.

Samples from Soil No. 2784, previously treated with sodium chloride, were retreated with one or more applications of calcium chloride, as indicated in table 3.

Friability measurements similar to those described, except that the drying process was interrupted at various stages and the cylinders were broken while they still contained moisture, were made for respective sodium- and calcium-treated samples (Soil No. 2784) in order to determine the relationship between the index of friability and the moisture content at breaking. Some of the samples were reground and remolded several times in order to obtain a variation in the moisture content at breaking (dry 110°C. soil = 0.0 per cent), which was determined by drying portions of the broken cylinders at 110°C. The relationship of moisture content at breaking to index of friability is shown graphically in figure 2.

Attempts were made to measure the physical properties for a bentonite (Soil No. 2788), but difficulty was encountered in drying the molded samples without their cracking. Both untreated and chemically-treated samples were used.

Replaceable bases were determined as previously described, for soils treated chemically. Replaceable sodium was determined for an untreated sample by subtracting the soluble sodium from the total amount removed during treatment with ammonium acetate. No attempt was made to determine replaceable calcium in the untreated soil because the solubility of calcium carbonate would be much lower at the beginning of the treatment when large amounts of soluble bicarbonate were present than later in the treatment when a portion of this anion had been removed. Data obtained are recorded in table 4.

DISCUSSION OF RESULTS<sup>4</sup>

The data presented herein, as well as observations made while obtaining these data, show that some physical properties of soils are altered materially by base exchange while others apparently are not.

During the washing process the maximum concentration of sodium chloride which permitted the dispersion of the sodium soil was higher than the maximum concentration of calcium chloride which permitted the dispersion of the calcium soil. It required about twice as long to wash samples treated with sodium as it did to wash calcium-treated samples, because of the failure of the highly

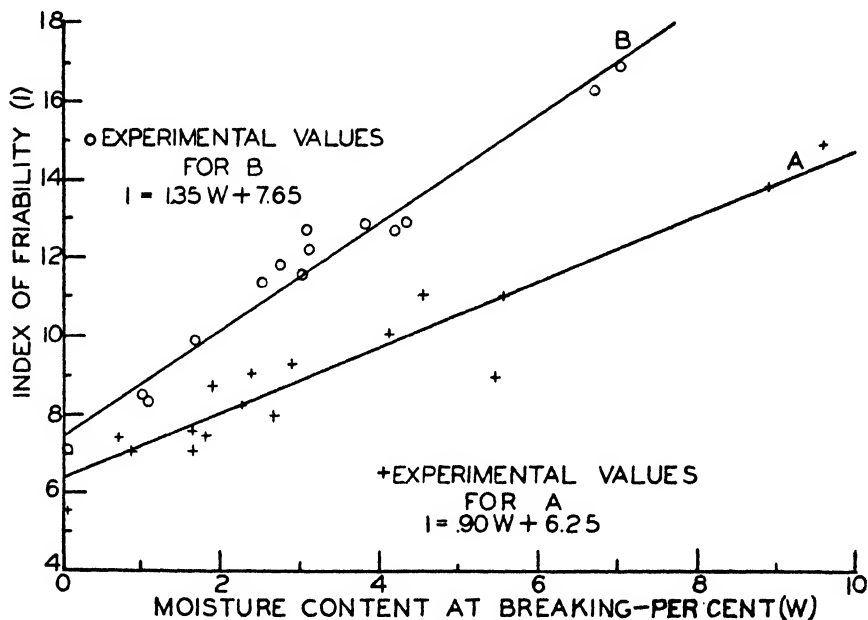


FIG. 2. RELATIONSHIP BETWEEN THE MOISTURE CONTENT AT BREAKING AND INDEX OF FRIABILITY FOR CHEMICALLY-TREATED SOILS. (A) SODIUM-TREATED SOILS. (B) CALCIUM-TREATED SOILS

dispersed sodium soil to settle below the filter as well as of low permeability to water. In all cases the first two washings from the sodium-treated samples were dark-colored. The dark color was probably due to some of the humus dispersoids going into colloidal solution, since the sodium humus dispersoids are more readily dispersed than the corresponding calcium dispersoids. Another difference between sodium- and calcium-treated soils was the greater tendency for the sodium-treated soil to crack during the process of drying. In the case of the bentonite used, the tendency to crack was so great that all

<sup>4</sup> A discussion of chemical equilibrium relationships of the replaceable bases will be included in a forthcoming publication by J. Darrel Peterson and D. S. Jennings.

samples were affected. Cylinders produced from sodium-treated bentonite, however, were more warped and distorted than those produced from calcium-treated bentonite.

TABLE 2  
*Physical properties of soils with one chemical treatment*  
(8 or 10 applications)

NO.		CHEMICAL APPLIED	MOISTURE FOR MOLDING	SHRINK- AGE COEFFI- CIENT	INDEX OF FRIA- BILITY	APPARENT DENSITY
Labora- tory	Substitute					
			<i>per cent</i>			
2784	Average	Untreated	22.6	17.61	5.79	1.973
2784	Av. for 9 samples	1 <i>N</i> NaCl	22.8	18.44	5.50	1.995
2784	Av. for 5 samples	1 <i>N</i> CaCl <sub>2</sub>	23.6	19.38	7.39	1.984
2784	15	0.25 <i>N</i> NaCl + 0.75 <i>N</i> CaCl <sub>2</sub>	22.2	18.46	8.25	1.974
2784	16	0.25 <i>N</i> NaCl + 0.75 <i>N</i> CaCl <sub>2</sub>	21.6	18.02	7.20	2.014
2784	6	0.5 <i>N</i> NaCl + 0.5 <i>N</i> CaCl <sub>2</sub>	21.2	17.97	7.81	1.999
2784	13	0.5 <i>N</i> NaCl + 0.5 <i>N</i> CaCl <sub>2</sub>	21.6	16.52	7.58	1.989
2784	12	0.75 <i>N</i> NaCl + 0.25 <i>N</i> CaCl <sub>2</sub>	22.1	17.92	7.96	1.986
2784	18	0.75 <i>N</i> NaCl + 0.25 <i>N</i> CaCl <sub>2</sub>	21.4	17.66	7.30	1.990
1867	1	1 <i>N</i> NaCl	22.0	.....	7.50	.....
1867	2	1 <i>N</i> KCl	24.0	.....	14.50	.....
1867	3	1 <i>N</i> CaCl <sub>2</sub>	25.0	.....	10.10	.....
1872	1	1 <i>N</i> NaCl	26.0	.....	7.64	.....
1872	2	1 <i>N</i> KCl	24.3	.....	11.20	.....
1872	3	1 <i>N</i> CaCl <sub>2</sub>	28.0	.....	10.90	.....
1872	4	1 <i>N</i> MgCl <sub>2</sub>	27.0	.....	10.90	.....

TABLE 3  
*Physical properties of sodium-treated soils treated subsequently with calcium*  
Soil No. 2784

SUBSTITUTE NO.	NUMBER APPLICA- TIONS OF CaCl <sub>2</sub>	MOISTURE FOR MOLDING	SHRINKAGE COEFFICIENT	INDEX OF FRIABILITY	APPARENT DENSITY
		<i>per cent</i>			
22	1	24.3	19.53	6.43	1.985
23	1	24.5	19.68	6.43	1.981
21	3	25.0	20.51	6.85	1.973
24	3	24.0	19.17	6.76	1.978
5	10	22.5	20.51	7.20	1.985

Since physical properties measured are interdependent, it is necessary to consider certain conditions or values constant, within experimental limits, when discussing the influence of replaceable bases upon them. The apparent density is the value considered constant throughout the following discussion.

Keen (7) shows that for a given soil there is a definite relationship between shrinkage coefficient and moisture content at time of molding. Puri (10) was unable to find any difference in the shrinkage coefficient (moisture content at time of molding being kept constant) when comparing sodium-treated soils with calcium-treated soils. The data presented in table 2 are, within experimental error, in agreement with this work. The data show that the relation-

TABLE 4  
*Replaceable bases in chemically-treated soils*  
(M.e. per 100 gm. of soil)

NO.		CHEMICAL APPLIED	REPLACEABLE BASES PRESENT	
Laboratory	Substitute		Ca	Na, K, or Mg
				Na
2784	AF	Untreated	.....	16.86
2784	7	1 N NaCl	10.60	6.90
2784	14	1 N NaCl	10.10	6.12
2784	25	1 N NaCl	10.50	5.56
2784	10	1 N CaCl <sub>2</sub>	17.18	0.98
2784	11	1 N CaCl <sub>2</sub>	17.70	0.47
2784	15	0.25 N NaCl + 0.75 N CaCl <sub>2</sub>	17.34	1.20
2784	6	0.5 N NaCl + 0.5 N CaCl <sub>2</sub>	16.47	1.51
2784	13	0.5 N NaCl + 0.5 N CaCl <sub>2</sub>	15.15	2.49
2784	12	0.75 N NaCl + 0.25 N CaCl <sub>2</sub>	16.26	1.42
2784	22	1st, 1 N NaCl -2d, 1 application CaCl <sub>2</sub>	17.85	0.30
2784	23	1st, 1 N NaCl -2d, 1 application CaCl <sub>2</sub>	17.85	0.13
2784	21	1st, 1 N NaCl -2d, 3 application CaCl <sub>2</sub>	17.10	0.18
2784	24	1st, 1 N NaCl -2d, 3 application CaCl <sub>2</sub>	16.48	0.16
2784	5	1st, 1 N NaCl -2d, 10 application CaCl <sub>2</sub>	18.33	0.43
1867	1	1 N NaCl	12.93	3.68
1867	3	1 N CaCl <sub>2</sub>	18.43	0.03
1872	1	1 N NaCl	3.72	12.19
1872	3	1 N CaCl <sub>2</sub>	15.02	0.02
				K
1867	2	1 N KCl	7.08	11.13
1872	2	1 N KCl	2.71	14.11
				Mg
1872	4	1 N MgCl <sub>2</sub>	0.72	17.73

ship between the moisture content at molding and the shrinkage coefficient is unaltered by base exchange. It may be that more refined measurements would show a difference.

An examination of table 4 shows that replaceable sodium was lower in sodium-treated Soils Nos. 1867 and 2784 containing calcium carbonate than in Soil No. 1872 containing practically no calcium carbonate, that samples treated with a mixture of sodium and calcium contained mainly replaceable



calcium, and that sodium-treated samples treated subsequently with one or more applications of calcium chloride contained practically no replaceable sodium. Kerr (8) and Vanselow (12) indicate that if a soil is treated with a mixture of salts, the resulting soil will contain a mixture of replaceable bases. The failure in this case to produce a soil containing both replaceable sodium and calcium and to saturate the soil with sodium may be accounted for by the presence of calcium carbonate in the soil. Samples treated entirely or partially with sodium chloride probably contained more replaceable sodium before the soluble salts were removed than after they had been eliminated. The calcium bicarbonate dissolved by the water from the soil during the washing process probably acted as a replacing agent and removed a portion or all of the replaceable sodium. In case of the sodium-treated samples treated again with one or three applications of calcium chloride, the replacement probably took place in two steps. A portion was replaced with the calcium chloride and the remainder with calcium bicarbonate.

The index of friability is dependent upon the chemical treatment of the soils (table 2). The soils treated with sodium had a lower index than soils treated with calcium. In case of Soil No. 2784, the average index of friability for the sodium samples was 5.50 and that for the calcium samples was 7.39, an increase of 1.89, or 34 per cent. Although this difference is not great, it shows that a sodium soil will be more difficult to plow or to cultivate than a calcium soil. The few determinations made for magnesium- and potassium-treated soils indicate an effect similar to calcium.

Certain properties of soils apparently depend upon previous treatment of the soil as well as upon the replaceable bases present. Volk (13) reports that potassium in potassium-treated soils can be changed from a replaceable to a non-replaceable form by wetting and drying the soil several times. Puri (10) found that if the sodium from sodium-treated soils were replaced by some other cation and the soil never allowed to dry, the latter would remain dispersed as though it were still sodium-saturated, but that if it were allowed to dry it would acquire the characteristics normally produced by the cation substituted. Burgess and Breazeale (1) apparently failed to recognize this fact when they reported that even small amounts of sodium would cause the soil to be dispersed. Harris (6) worked with soils in which the difference in replaceable base content was produced naturally in the fields where the soil had been subject to wetting and drying several times; consequently, he was able to find a relationship between percentage of replaceable sodium and permeability to water. The concentration of the treating solution seems to determine to some extent the physical properties of the soil after treatment. In this laboratory a sodium-treated bentonite treated again with 0.1 *N* ammonium acetate remained dispersed even after the replaceable sodium was all removed, but when solid ammonium acetate was added to the sodium-treated bentonite first and water added in small portions it remained flocculated throughout the entire treatment.

The theory of influence by previous treatment probably will explain the fact that the index of friability corresponds more closely to the treatment of the soil than to the replaceable bases present in the soil after treatment (tables 2, 3, and 4). The index found is probably dependent upon the replaceable sodium in the soil before washing and not upon that in the soil after washing. The removal of the replaceable sodium by dilute calcium bicarbonate while the soil is dispersed leaves the physical properties of the soil comparatively unchanged, but the removal of replaceable sodium with normal calcium chloride while the soil is flocculated produces a change in physical properties. This will account for the fact that the variation in index of friability for sodium- and calcium-treated soils for Soils Nos. 1867 and 2784 is about the same as that for Soil No. 1872 from which the replaceable sodium was not removed by calcium bicarbonate. It will also explain why certain sodium-treated samples (Soil No. 2784) treated subsequently with calcium chloride possessed an index of friability in between that sodium- and calcium-treated samples. The amount of sodium removed by calcium chloride will depend upon the number of applications; therefore, the samples again treated with the smallest number of applications should have the lowest index of friability. An examination of tables 2, 3, and 4 shows this to be the case. Substitute Nos. 22 and 23, again treated with one application of calcium chloride, had a lower index of friability than Substitute Nos. 21 and 24 again treated with three applications, whereas Substitute No. 5, again treated with ten applications, had an index similar to the calcium-treated soils. This theory fails to show, however, why the samples treated with a mixture of sodium and calcium should have the highest index of friability. This, however, may be due to errors made in making measurements of index of friability.

Data obtained (fig. 2) indicate that the difference between the index of friability for calcium-treated and sodium-treated samples (Soil No. 2784) is greater when the soil contains some moisture than when the soil is dry. The index increases with increasing moisture content for both types of soil, but the increase is greater for calcium soil. Both relationships are apparently straight lines. The equation for the sodium soil was  $I = .90 W + 6.25$  and that for the calcium soil was  $I = 1.35 W + .65$ , where  $I$  equals index of friability and  $W$  equals moisture content at breaking. It was impossible to obtain data for samples with more than 10 per cent moisture because, instead of breaking under pressure, the soil cylinders had a tendency to flatten.

#### SUMMARY

A study was made of the influence of replaceable bases on various physical properties of soils, with special reference to the influence of replaceable calcium and sodium on the index of friability.

Modifications are reported in Christensen's method for determining the friability of soils. The determinations reported in this study indicate that treating the soil with sodium lowers the index of friability, thus producing a soil inferior in physical condition to calcium-treated soils.

The difference between the index of friability of a sodium-treated and a calcium-treated soil is greater for the wet than for the dry soil.

It was found that the index of friability of soils corresponds more closely to the chemical treatment than to the replaceable bases present after treatment. The theory advanced to explain this fact assumes that the physical properties of sodium soil are changed if the replaceable sodium is removed by high salt concentration, such as a normal solution of calcium chloride, whereas the physical properties are comparatively unchanged if the sodium is removed by dilute solutions, such as calcium bicarbonate formed from calcium carbonate in the soil.

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# STUDIES ON THE SURFACE BEHAVIOR OF BENTONITES AND CLAYS<sup>1</sup>

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Application of electrostatics has met with considerable success in the explanation of the behavior of systems the components of which possess marked electrical character. The interaction of clays with water and aqueous solutions of electrolytes where the acting agents are charged surfaces, ions, and dipoles should, therefore, be mainly a function of their electrical character. The main difficulty in the application of electrostatics to the explanation of the actual behavior of clays toward water and ions lies in the indeterminacy of the structure of the electric field at the surface of the colloidal particle. Obviously, the best application that can be made of the theory is to calculate the behavior of clay-water systems on the basis of logical assumptions and to accept as facts those assumptions which lead to the best mathematical description of the observed phenomena.

Duclaux (3) has calculated the distribution of ions around a spherical particle of opposite charge. His equation, the derivation of which will be brought out later in this paper, shows that the thickness of the ionic atmosphere around a colloid particle is of the same order of magnitude as the particle itself. Consequently, the Duclaux formula can be expected to hold only when the colloidal particle possesses a spherical shape and uniformly distributed charge. In the case of soil colloids where there is no *a priori* reason why they should have any particular form, and where usually both cation and anion exchanges are found, this equation cannot be expected to possess general applicability.

The physico-chemical behavior of the surface of a colloidal particle is a function of both the geometrical and electrical properties of this surface. From a geometrical viewpoint the surface may be of the convex, plane, or concave type. Electrically, it may represent a sparse to dense system of point charges of either the same or of opposite sign. In the following, equations are given for the ideal convex surface (sphere) as well as for the plane surface type, developed under the assumption of a uniform and, for the plane type, relatively dense surface charge. The influence of variations in the electrical character will be treated later.

<sup>1</sup> Published with the approval of the department of soils, University of Missouri, Columbia, Missouri, and of the Missouri State Highway Department, Jefferson City, Missouri.

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The equation for the distribution of ions around a charged sphere has been developed by Duclaux as follows:

- If
- $e$  = the elementary electric charge
  - $P$  = the number of the swarm ions
  - $p$  = the charge of the swarm ions
  - $N$  = the Avogadro number
  - $c$  = the number of ions per unit volume
  - $r$  = the distance from the center of the granule
  - $dr$  = an increment of distance
  - $ds$  = an element of surface
  - $K$  = the dielectric constant of the liquid medium
  - $R$  = the gas constant
  - $T$  = the absolute temperature

then the electrostatic attraction of the granule on the ions contained in the volume element  $ds \cdot dr$  is

$$E = \frac{Pp^2e^2cdsdr}{Kr^2}$$

and the resultant of the osmotic pressures on the two sides of the volume section  $ds \cdot dr$  is

$$O = \frac{RT}{N} \cdot \frac{dc}{dr} \cdot ds \cdot dr$$

therefore, in the state of equilibrium

$$\frac{Pp^2e^2cdsdr}{Kr^2} = \frac{RT}{N} \frac{dc}{dr} \cdot ds \cdot dr$$

which on integration gives

$$c = Ae^{\frac{Pp^2e^2N}{KRT} \cdot \frac{1}{r}}$$

A plane surface structure is defined as one in which charge points of the same sign are densely and uniformly spread over a plane surface. In this case the Faraday lines will run parallel in a right angle from the surface and will end in the oppositely charged ions. Figure 1 shows pictures for the convex and for the plane surface field.

The equation for the electrical attraction in the case of the plane field structure is then

$$E = \frac{Pp^2e^2cdsdr}{Kr}$$

\*  $e$ , basis of natural logarithms.

and for the equilibrium of osmotic and electrical forces,

$$\frac{Pp^2e^2cdsdr}{Kr} = \frac{RT}{N} \frac{dc}{dr} \cdot ds \cdot dr$$

$$dc = \frac{NPp^2e^2}{KRT} \frac{dr}{r}$$

or if

$$\frac{NPp^2e^2}{KRT} \text{ is kept constant } = k$$

$$\ln c = K \ln r + A$$

$$c = Br^k$$

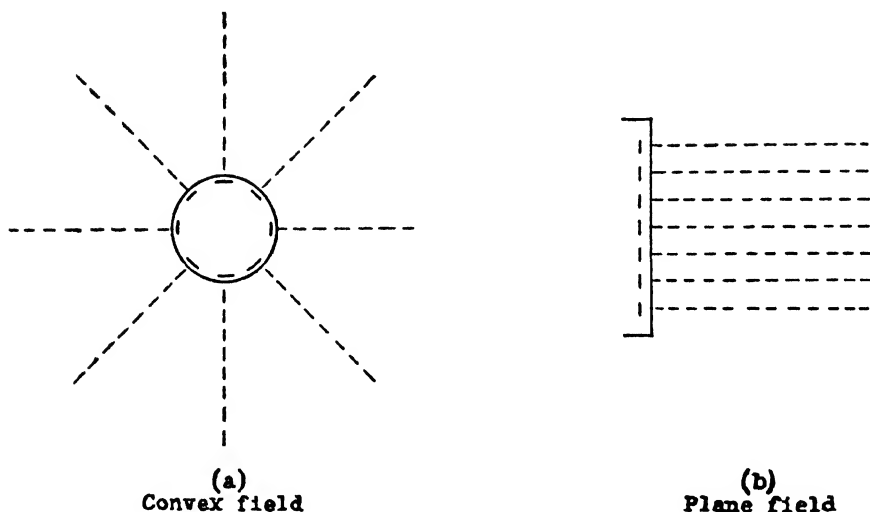


FIG. 1. ELECTRICAL SURFACE STRUCTURE OF COLLOIDAL PARTICLE

Calculations on concentrations of mono, bi, and trivalent ions at different distances from the surface of a spherical granule of  $2 \text{ m}\mu$  radius and with 12 negative charges are given in table 1. The physical magnitudes used in the calculations are:

$$e = 4.8 \cdot 10^{-10} \text{ electrostatic units}$$

$$N = 6 \cdot 10^{23}$$

$$K = 80$$

$$RT = 2.38 \cdot 10^{10} \text{ erg}$$

After insertion of the numerical values in the equilibrium equation for the plane shaped particle it becomes apparent that  $k$  is approaching zero sufficiently to render  $r^k = 1$ , and  $c = B$ .

An analysis of the equations for the convex and for the plane field shows

that in the first case the ionic concentration is very high close to the surface and falls off rapidly with increasing distance. This decrease becomes more rapid with increasing charge of the ions. In the case of the plane surface the concentration stays practically constant with increasing distance from the surface. It should be kept in mind, however, that these data are calculated

TABLE 1  
*Distribution of ions around a spherical colloidal particle (3)*

DISTANCE FROM SURFACE  <i>mμ</i>	CONCENTRATIONS		
	C <sub>1</sub> Monovalent ions	C <sub>2</sub> Divalent ions	C <sub>3</sub> Trivalent ions
0	63 A <sub>1</sub>	40 A <sub>2</sub>	25 A <sub>3</sub>
1	16 A <sub>1</sub>	2.5 A <sub>2</sub>	0.40 A <sub>3</sub>
2	8 A <sub>1</sub>	0.63 A <sub>2</sub>	0.05 A <sub>3</sub>
4	4 A <sub>1</sub>	0.32 A <sub>2</sub>	0.003 A <sub>3</sub>
8	2.3 A <sub>1</sub>	0.05 A <sub>2</sub>	0.001 A <sub>3</sub>
30	1.2 A <sub>1</sub>	0.02 A <sub>2</sub>	0.0002 A <sub>3</sub>

A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub> are constants.

TABLE 2  
*Physico-chemical properties of some colloidal clays*

PROPERTY	TYPE OF H COLLOID					
	Benton- ite	Lufkin clay	Wabash clay	Putnam clay	Susque- hanna clay	Cecil clay
SiO <sub>2</sub> -R <sub>2</sub> O <sub>3</sub> ratio.....	5.0	3.8	3.2	3.2	2.3	1.3
Exchanged capacity, m.e./gm.....	0.95	0.82	0.78	0.65	0.47	0.13
Swelling,* cc./gm.....	2.20	1.18	0.94	0.81	0.57	0.05
Heat of wetting, cal./gm.....	(16.0)	15.0	13.9	13.8	11.7	5.9
Hygroscopicity (30 per cent H <sub>2</sub> SO <sub>4</sub> ), per cent by weight.....	21.5	20.1	.....	18.1	15.5	6.1
Swelling, cc.....	(0.14)	0.08	0.07	0.06	0.05	0.01
Heat of wetting, cal./gm.....	2.44	1.44	1.20	1.24	1.21	0.41
Swelling, cc.....	(16.8)	18.3	17.8	21.2	24.9	46.4
Exchange capacity, cal./gm.....						
Heat of wetting.....						
Exchange capacity.....						

\* Measured by the Winterkorn-Baver method.

for an ideal case. Actually, an ideal plane field will never be encountered on the surface of the colloidal granules. Moreover, if it would exist close to the surface it would be dispersed with increasing distance, because of the limited size of the plane as well as the fact that the electrical force lines end in the swarm ions.

It is obvious that, if the active volume of the colloidal particle consists of the volume of the granule and of the denser part of the ionic atmosphere, a larger active volume in the case of a plane field structure will be found than in the case of a spherical particle shape. Since in the aforementioned derivations the repulsive action of the ions composing the atmosphere has been neglected, the values for the active volumes of the particles calculated by means of these equations represent minimum values. It should be further kept in mind that the dielectric constant of water approaches 3 in very strong

TABLE 3  
*Data on swelling and dispersity of clays and bentonite*

TYPE OF CLAY	SiO <sub>2</sub> -R <sub>2</sub> O <sub>2</sub>	SWELLING, CC./GM.					
		H	Li	Na	K	Ca	Ba
Bentonite.....	5.0	2 22	10.8	11.1	8.6	2.5	2.5
Putnam.....	3.2	0.81	5.0	4.0	0.5	0.91	0.85
Wabash.....	3.2	0.94	3.1	3.7	0.55	0.79	0.74
Iredell.....	1.8	0 23	0 42	0.6	0 02	0 30	0.36

<i>Dispersity &lt; 100 mμ</i>							
Bentonite.....	5.0	34 0	37.8	35.2	35.7	31.2	31.9

TABLE 4  
*Symmetry values of clays and bentonites*

TYPE OF CLAY	NATURE OF CATION					
	Li	Na	K	Ca	Ba	H
NH <sub>4</sub> ·Putnam.....	29.9	35.3	51.3	63.6	71.6	84.9
H·Putnam.....	6.6	6.2	14.5	26.9	23.8	....
H·Bentonite.....	18.0	14.6	18.4	....	....	....

<i>Release</i>						
NH <sub>4</sub> Cl + Putman clay.....	57.0	58.9	42.0	29.3	....	6.9
NH <sub>4</sub> Cl + Bentonite.....	46.5	49.6	43.7	....	....	14.5

electric fields. Another important point which has been neglected is the hydration of the ions, or, more generally, the polarization effects in the system.

Beside the rôle that they play in the hydration of the ions and of electrically charged surfaces, polarization effects appear also to be responsible for the anomalies in the exchange of bivalent ions. For example, with monovalent ions easy adsorption corresponds with difficult release; on the other hand, with divalent ions easy adsorption and easy release go parallel. For a complete electrostatic analysis of this problem energy calculations would have to be made for a system for the cases: (a) that the cation is entirely hydrated and is found as close to the negative charge as its hydration hull permits; (b) that



the cation is completely dehydrated and that besides the coulombic attraction of the ion and the oppositely charged surface, attraction due to polarization plays a significant rôle; and (c) that the cation is dehydrated only at the side adjacent to the negative charge, and that the energies of the partial hydration, of the ionic attraction, and of the polarization effects come into play. The data needed for quantitative information on the probability of these three conditions represent relatively small differences of rather large magnitudes; unfortunately they cannot yet be determined or calculated accurately enough to be used as a convincing argument. Nevertheless, a qualitative explanation is rather easily conceived. If we assume, as seems logical, that the negatively charged oxygen at the colloidal surface which is held by part of the positive charges of a silica ion below the surface is in a similar electric condition as the oxygen in a hydroxyl ion, then the magnitude of the polarization effect of the bivalent ion on the surface oxygen should be similar to that on the hydroxyl ion. Consequently, the ease of release of such a cation from a colloidal surface should run parallel to the ease of dissociation and, therefore, to the solubility of its hydroxide in water. This has been shown experimentally (5).

With the exception of the case where the polarization of the surface of a colloidal granule by a cation would result in a greater decrease in free energy than is connected with the hydration of the ion, this ion can approach the surface only as closely as its hydration permits. In the case of spherical granules, where the ionic density is very large close to the surface, it should be expected that the increase in apparent volume in a liquid is largely governed by the solvation of the ions, and that swelling is, therefore, closely connected with the heat of wetting (4).

Because of the greater proximity of the majority of the swarm ions to the surface of a spherical particle, the individual properties of the ions, such as size, charge, polarizability, and polarizing power, should exert a more pronounced influence on the base exchange properties of such a colloid than on the properties of a colloid with plane surface structure.

Plasticity studies and microscopic examinations of oriented aggregation of clays indicate that most of the common clays possess a geometrical plane structure. On the other hand, studies of their colloidal properties show that they possess cation and anion exchange capacities, the latter increasing with decreasing  $\text{SiO}_2\text{-R}_2\text{O}_3$  ratio of the colloid. It appears worthwhile, therefore, to discuss generally in which direction a heterogeneously charged system will change the properties as derived from the assumption of a plane surface with uniform charge.

If the surface represents a system of positive and negative points, and if the ionic atmosphere consists of negative and positive ions, then the decrease of the strength of the surface field will be a function of a power of the distance higher than the first; thus, the character of the ion atmosphere will approach that of the swarm around a spherical granule. This tendency to greater compactness of the ionic atmosphere will be fostered by a decrease in the repulsion

of ions of the same kind of charge due to the presence of those with opposite sign. On the other hand, positive surface charges which are situated close to a negative charge will counteract the attraction of a swarm cation by this charge (*et vice versa*). This will effect a better exchangeability, especially in the case of those ions which are generally held very strongly at the surface (6).

Table 2 shows experimental data on the properties of different clays (2). Some interesting relationships are brought out. In the light of the theory developed in the foregoing it appears especially important that, although the ratio of heat of wetting to exchange capacity for bentonite, Lufkin, and Wabash clay indicates that the H ions are in the same state of hydration, the ratio of swelling to exchange capacity shows that in the case of bentonite the ion atmosphere is more diffuse. Bentonite, therefore, appears to be representative of clays with the plane type of surface structure. Another interesting fact is the increase of the ratio of heat of wetting to cation exchange capacity and the decrease of the ratio of swelling to heat of wetting and of swelling to cation exchange capacity with decreasing  $\text{SiO}_2\text{-R}_2\text{O}_3$  ratio. It is generally known that the anion exchange capacity of clays increases with decreasing  $\text{SiO}_2\text{-R}_2\text{O}_3$  ratio, i.e., with the decrease of this ratio the number of positive charges in the surface increases. The foregoing data as well as those of other investigators (4) suggest that, because of their connection with relatively large silica-oxide complexes, the negative charges are situated too far inside the surface to bind water with any appreciable thermal effect. Positive charges, on the other hand, originating from iron and alumina in the surface seem, according to these data, to be able to orient and eventually polarize water molecules. This view is further strengthened by the observations of Bayer and Horner (1) that with decreasing cation exchange capacity and with decreasing  $\text{SiO}_2\text{-R}_2\text{O}_3$  ratio soil colloids absorb less water at low but retain more at high temperatures.

After the identification of bentonite as representative of the clays with plane type of surface structure and of the common clays as approaching in their behavior that of the convex type of surface, it should be expected that the individual characteristics of the swarm ions affect the properties of clays more than they affect those of bentonites. This view is confirmed by the data in tables 3 and 4 (2).

With bentonite the swelling is of the same order of magnitude whether the exchange complex be saturated with Li, Na, or K; however, with clays the swelling in the case of K saturation is much smaller than with the Li and Na saturation. The similarity of the effect of Li, K, and Na ions on bentonite is also shown by the dispersity data. The same relationship holds true in the case of the symmetry values.

On the assumption of a plane surface structure for bentonite, its thixotropic character is also readily explained. Since the preceding rather elementary application of electrostatics resulted in the explanation of the general difference in the behavior of clays and bentonites, it is felt that further consequent use of electrostatics will help in elucidating still other phases of the surface behavior of these substances.

## SUMMARY

An attempt has been made to explain the general difference in the behavior of bentonites and clays on the basis of the electrical properties of their surfaces. Experimental data are given in support of the theoretical conclusions.

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# NOTES ON ESTIMATING AVAILABLE PHOSPHORUS BY EXTRACTING SOILS WITH A POTASSIUM CARBONATE SOLUTION

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Das in 1926 (1) and again in 1930 (2) described a method of estimating available phosphorus in soils by extracting with a dilute potassium carbonate solution. His results showed that the method was equally applicable to all types of soil and that it had the advantage over acid extraction methods in that it could be used in alkaline calcareous soils where acid extraction methods were not reliable. Hockensmith et al. (3) in 1932 found that a modification of the Das method, consisting of a hot rather than a cold extraction, distinguished between phosphate deficient and sufficient soils in Colorado in a high percentage of cases.

Since there is a large area of alkaline calcareous soils in the western United States where acid extraction methods are not reliable and since there is a great need of a reliable method in this area, the authors believe that the use of an alkaline extractant deserves further study by soil investigators. It is with the hope of stimulating further investigation that the following procedure is submitted. The procedure as originally published by Hockensmith et al. had the disadvantage that the color faded quickly and was often cloudy so that accurate determination of the phosphorus was difficult. The revised procedure gives a color which is usually stable for several hours and is seldom cloudy. The method of determining the phosphate in the potassium carbonate extract is also applicable to other phosphate solutions.

## PROCEDURE

Weigh out a 2-gm. sample of soil, which has been passed through a 1-mm. sieve, and place in a 250-cc. flask. Add 150 cc. of a 1 per cent solution of phosphorus-free potassium carbonate and place on a previously heated hot plate. The temperature should be regulated so that the solution simmers for about 45 minutes. In 60 minutes,<sup>1</sup> remove the flask and allow it to cool. After settling for 12 to 24 hours,<sup>2</sup> transfer 10 cc. of the supernatant liquid to a 100-cc. Erlenmeyer flask. Add 1 cc. of bromine water. Heat to boiling,<sup>3</sup> and add

<sup>1</sup> It was found in the laboratory that digestion for less than an hour decreases the phosphorus content. However, prolonged digestion affects the phosphorus but little.

<sup>2</sup> The cooled sample may be centrifuged to obtain a clear solution if speed is essential.

<sup>3</sup> This may be accomplished conveniently on an electric hot plate. The solution should not be allowed to evaporate to dryness.

0.4 cc. of 5 *N* HCl, and 0.5 cc. of 10 per cent sodium sulfite. The extract should be clear at this point. Remove and cool. Neutralize (1 drop in excess) with 0.5 *N* NaOH, using 1 drop of 0.25 per cent alcoholic phenolphthalein as the indicator. If the end point is over-run, titrate back with 0.5 *N* sulfuric acid. Make up to 15 cc. with distilled water and shake.

To the slightly pink solution, add 1 cc. of the molybdic oxide reagent from a burette. Shake, add exactly 0.2 cc. of stannous chloride reagent, and mix well. After 5–10 minutes,<sup>4</sup> place 10 cc. of the solution in a square test tube and compare the color with the standard phosphate solution, using any good color comparator.

### Solutions

Potassium carbonate—1 per cent by weight  $K_2CO_3$ . Of this solution 150 cc. should be drawn off and tested for freedom from phosphorus by the foregoing method.

Bromine water—saturated.

Hydrochloric acid—approximately 5 *N*.

Sodium sulfite—10 per cent—C.P. grade. Make up fresh about every 2 weeks.

Sodium hydroxide—approximately 0.5 *N* NaOH.

Sulfuric acid—approximately 0.5 *N*  $H_2SO_4$ .

Molybdic oxide reagent (4)—add 6.02 gm. C.P. anhydrous  $MoO_3$  to 120 cc. of concentrated  $H_2SO_4$  (sp. gr. 1.84:94 per cent). Heat with stirring until dissolved. Cool and make up to 800 cc. with distilled water.

Stannous chloride—stock solution. Dissolve 25.5 gm.  $SnCl_2 \cdot 2H_2O$  in 125 cc. of HCl (sp. gr. 1.18:35–7 per cent) and make up to exactly 1 liter. Keep under white mineral oil in a dark bottle. If kept in a cool place, this solution will keep indefinitely.

Work solution. Quantitatively dilute 50 cc. of the stock solution to 250 cc. (dil. 1–5) with pure distilled water. This solution must be renewed every 2 or 3 days, or when it turns yellow.

When the foregoing procedure is used, 37 parts phosphorus extracted per million parts soil are equivalent to 55 p.p.m., by the procedure of Hockensmith et al. (3). This has been shown to be approximately the dividing line between deficient and sufficient soils for the soils studied.

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<sup>4</sup> The color will be very nearly constant after standing for 5 minutes. The intensity will not change enough to be detected for at least 15–20 minutes. However, it is better to make the comparison after 5–10 minutes.

# SHOT SOILS OF WESTERN WASHINGTON<sup>1</sup>

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Most of the upland soil types of western Washington, a humid region west of the Cascade mountain range, are characterized by the presence of a considerable number of hard, rounded pellets distributed throughout the developed portion of the soil profile. These pellets vary from the dimensions of very fine sand to the size of marbles. They are generally rounded spheroids with a rough surface upon which small protuberances may occur. The proportion of such pellets is so large in many soils that agriculturists have applied the term "shot clays" to them. Bennett and Allison (1) called attention to the presence of iron pellets, locally called "perdigon," in many of the lateritic clays of Cuba. An investigation of the occurrence and of some of the properties of the shot soils of Washington has been undertaken because of the genetic as well as practical interest attached to the process of shot formation.

## OCCURRENCE OF SHOT SOILS

Field observations have revealed the presence of shot in upland soils developed from both glacial and residual material. The pellets form under forests but are absent under prairie conditions. Shot are found in soils belonging to both the brown and gray forest soil groups but are absent in forested lowland soils where poor drainage occurs and are not generally found in soils that are excessively drained. The most abundant formation of shot occurs in soil with restricted internal drainage but where the movement of water in the soil is essentially downward. Where shot occur, the soils are invariably acid in reaction. Shot appear to be a natural development in the normal soil profile of the region.

## CLIMATIC CONDITIONS OF WESTERN WASHINGTON

Records available in the region of shot formation indicate that the pellets form under an annual rainfall ranging from more than 120 inches to less than 20 inches. The exceptionally mild winter temperatures in the Puget Sound country allow an almost constant winter percolation to occur in upland soils. This contributes to the formation of well-leached, mature profiles. A dry period usually occurs near the middle of the summer season, which is signifi-

<sup>1</sup> Published as Scientific Paper No. 318, College of Agriculture and Experiment Station, State College of Washington.

cant because it brings about strong precipitation and dehydration processes within the soil and perhaps a reversal in the direction of water movements within the profiles.

The range in mean annual temperatures in localities below 1000 feet in elevation is small. While the mean for 48 stations averages 48.1°F., the variation from this mean is only about 3° either way. It seldom becomes extremely hot or extremely cold, the mean maximum temperature being about 58°F. and the mean minimum temperature about 38°F. The region is characterized by high relative humidities generally in the neighborhood of 80 per cent, although on summer afternoons it has been found to drop to 52 per cent. The average relative humidity of the air at all Puget Sound weather stations is 83 per cent.

#### FOREST COVER OF WESTERN WASHINGTON

The moist climate with relatively warm temperatures produces a heavy forest growth over practically all of western Washington. The most widely distributed tree is the Douglas fir (*Pseudotsuga taxifolia*), which makes up from 90 to 100 per cent of the forests up to an elevation of 1000 feet. In a narrow strip near the ocean, however, Sitka spruce (*Picea sitchensis*) is the dominant tree of the uplands. Associated with the Douglas fir, especially in local areas with slightly more than average ground moisture, are the true firs, lowland white fir (*Abies grandis*) and silver fir (*Abies amabilis*). Western hemlock (*Tsuga heterophylla*) occurs at higher elevations and in addition is often found occupying the more open areas where the Douglas fir has temporarily been eliminated because of natural causes. This replacement occurs because hemlock seedlings grow well in the rotten wood of fallen trees, the roots following the old tree trunk and completely permeating it. Seedlings of Douglas fir do not do this. In swampy places and on lower slopes where seepage occurs the Western red cedar (*Thuja plicata*) grows luxuriously. On many of the drier gravel plains lodgepole pine (*Pinus contorta*) establishes itself in nearly pure stands. On certain of the better soil types and especially where more than average amounts of organic matter are present in the soil, almost pure stands of red alder (*Alnus rubra*) occur. Cottonwood (*Populus trichocarpa*) may become thickly established in the river bottoms.

When the stand of Douglas fir is dense and well developed, scarcely any vegetative ground cover exists other than mosses and ferns, but when more light reaches the ground, a luxurious ground cover of shrubs and herbaceous plants is developed. Oregon grape (*Berberis aquifolium*), sallal (*Gaultheria shallon*), and huckleberry (*Vaccinium ovalum*) are the most common shrubs. The bracken fern (*Pteridium aquilinum pubescens*) is also widely distributed. Many species of mosses occur.

In spite of the luxurious forest growth there is a disappointingly small accumulation of forest mold. In certain old established Douglas fir forests known to be over 300 years old and presumably untouched by fires the mold

is only 1 or 2 inches thick. It is acid in reaction and relatively low in basic material.

#### PHYSICAL STUDIES OF THE SOILS

##### *Quantity of shot in soils*

Samples of the horizons of several soil profiles in western Washington were examined in the field during the dry summer season. The material was screened through a 2-mm. sieve with round holes. Gentle pressure with a rubber pestle was employed to break up the soil granules and to crush everything except the coarse gravel and the shot. After the coarse gravel was separated from the shot by visual selection, the three fractions were weighed. The results are presented in table 1.

The results show that there is no definite zone in all profiles where the greatest formation of shot takes place, although in three glacial soils the maximum development occurs in the region from 8 to 20 inches. One soil shows a maximum development in the 3-12 inch layer and one in the surface layer. In the latter case, however, conditions were not conducive to strong shot formation. In the residual soils the maximum shot development was found in the upper layers with decreasing numbers below a depth of 12 inches.

In these profiles, the greatest accumulation of shot occurs in the Kingston loam, which is underlaid by dense, impervious, brownish gray glacial drift. The weakest shot formation occurs in the Everett sandy loam soil, which is underlaid by open stratified gravel beds. These observations indicate that there is a stronger tendency for shot to form when the water movements within the profile are restricted.

Because of the marked similarity in forest and vegetative ground cover where these profiles were examined, no indications regarding the effect of these factors could be observed. The almost identical reaction, as indicated by similar pH values as determined in the field, implies that this factor is not a satisfactory explanation for differences in intensity of shot development.

The variation in size of the larger shot is indicated in figure 1 of plate 1, in which the largest individual measures about 19 mm. in diameter. The appearance of the larger shot, as they are embedded in the matrix of soil, is illustrated in figure 2 of plate 1, in which the three lumps from left to right represent the surface, subsoil, and clay substratum horizons in a profile. The granular character of the upper two horizons, due to the presence of these pellets, is clearly indicated. Figure 3 of plate 1 shows the effect of the rain in a road cut through a shot-bearing silt loam soil. The pellets protect the soil beneath them from washing and appear as caps on the soil columns left intact.

##### *Mechanical composition*

Representative samples of shot of all sizes larger than 2 mm. in diameter were selected from weathered horizons of several profiles and subjected to a long continued treatment with weak hydrochloric acid on a steam bath to break



TABLE 1

*Percentage of shot and of coarse gravel larger than 2 mm. in diameter present in different horizons of several soil profiles in western Washington*

The last horizon in each case is parent material

SOIL TYPE	HORIZON	DEPTH	SOIL	SHOT	COARSE GRAVEL
<i>Glacial group</i>					
		<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Kitsap silt loam.....	1	0-8	84.4	15.6	0.0
	2	8-20	69.6	30.4	0.0
	3	20-	99.5	0.0	0.5
Alderwood loam.....	1	0-3	58.0	12.0	30.0
	2	3-10	49.0	26.0	25.0
	3	10-20	52.0	29.0	19.0
	4	20-28	56.0	25.0	19.0
	5	28-	58.0	0.0	42.0
Alderwood sandy loam.....	1	0-3	67.0	30.0	3.0
	2	3-12	60.0	33.0	7.0
	3	12-24	61.0	27.0	12.0
	4	24-	52.0	0.0	48.0
Kingston loam.....	1	0-2	65.0	27.0	8.0
	2	2-8	33.0	50.0	17.0
	3	8-20	32.0	65.0	3.0
	4	20-28	77.0	0.0	23.0
	5	28-	50.0	0.0	50.0
Everett sandy loam.....	1	0-4	59.0	2.0	39.0
	2	4-12	48.0	1.0	51.0
	3	12-24	40.0	0.0	60.0
	4	24-30	50.0	0.0	50.0
	5	30-	34.0	0.0	66.0
<i>Residual group</i>					
Olympic clay loam.....	1	0-8	53.0	47.0	0.0
	2	8-20	54.0	46.0	0.0
	3	20-30	65.0	31.0	4.0
	4	30-42	98.0	0.0	2.0
	5	42-	90.0	0.0	10.0
Wind River loam.....	1	0-4	60.0	40.0	0.0
	2	4-12	62.0	38.0	0.0
	3	12-20	65.0	35.0	0.0
	4	20-36	70.0	30.0	0.0
	5	36-	89.0	0.0	11.0
Underwood loam.....	1	0-4	51.0	49.0	0.0
	2	4-12	46.0	54.0	0.0
	3	12-24	71.0	29.0	0.0
	4	24-36	94.0	6.0	0.0
	5	36-	100.0	0.0	0.0

them down. A concentration of about 3 per cent of acid was required before disintegration occurred, and from 1 to 8 per cent of the original weight of the shot was lost through solution processes. After complete breakdown the acid was filtered off, the residue washed with water and then placed in a dispersion machine together with an excess of sodium hydrate solution. After dispersion the percentages of the various soil separates were determined by the hydrometer method of Bouyoucos (2) and by screens. Samples of the soils

TABLE 2

*Mechanical composition of the disintegrated shot larger than 2.0 mm. in diameter and of the corresponding soil from which the shot were screened*

SOIL	HORIZON AND SAMPLE	PERCENTAGE IN DIFFERENT SIZE GROUPS (DIAMETERS IN MM.)							
		>2.0	2.0-1.0	1.0-0.5	0.5-0.25	0.25-0.10	0.10-.005	0.5-0.05	0.005-
Kitsap silt loam.....	1 shot	1.35	2.01	3.55	5.65	13.77	17.57	25.30	31.05
	2 shot								
	1 soil	0.00	1.69	4.87	2.03	13.54	11.72	16.10	50.05
	2 soil	0.00	1.79	4.91	2.58	9.68	16.89	20.20	43.95
Kingston loam.....	1 shot	0.00	0.49	3.73	3.65	9.84	9.56	57.11	15.62
	1 soil	8.00	1.26	4.97	6.86	31.04	24.09	16.83	14.25
Clallam sandy loam.....	1 shot	38.40	3.15	3.78	4.10	10.84	4.70	50.11	23.32
	1 soil	20.00	9.28	16.32	8.50	14.23	7.57	28.02	16.08
	2 shot	26.80	7.80	9.04	7.21	9.79	8.80	36.54	20.82
	2 soil	18.00	8.02	16.18	10.10	16.05	8.75	24.02	16.88
Alderwood sandy loam.....	2 shot	23.00	1.76	4.83	4.70	14.23	36.10	16.56	19.85
	2 soil	7.00	9.14	11.36	5.52	19.75	20.88	17.64	15.70
	3 shot	20.0	3.06	4.50	3.97	13.45	26.65	31.47	16.90
	3 soil	12.0	11.22	12.72	7.29	16.26	15.68	22.14	14.81
Wind River loam.....	2 shot	3.20	0.40	4.22	6.94	24.74	31.88	19.14	12.68
	2 soil	0.00	2.82	8.17	6.64	26.49	10.51	22.96	22.40

which surrounded the shot in each horizon were similarly analyzed. The results of these studies are presented in table 2.

The analyses bring out an interesting characteristic of the shot material by showing that coarse gravel particles may act as nuclei for shot formation. In two samples where coarse gravel was naturally present in the soil, a larger amount occurs in the disintegrated shot; and in two cases where the soil contained no gravel the shot, when broken down, released such particles. One soil, of those analyzed, behaved in exactly the opposite manner. A large pro-

portion of the shot from the Alderwood sandy loam soil had gravel nuclei, whereas the shot from the Kingston loam soil were entirely free from them. These coarse particles apparently became cemented into the pellets and were again released during the acid digestion.

Microscopic observations during the screening of the sand fractions indicated that shot occur in the fine sand separates from all the weathered horizons but do not appear in the very fine sand fraction. This indicates that shot may also start as small aggregates and apparently grow as weathering proceeds.

In five of the eight horizons examined, the disintegrated shot contained more of the silt separate than the corresponding soil. In the other three horizons there was more clay in the disintegrated shot than in the soil. It is possible that the acid digestion used to break up the shot may have influenced this, but since the solution processes mainly attacked the cementing material of the shot aggregates, some indication is given of weathering trends since the time of cementation of the shot.

#### CHEMICAL ANALYSES

Samples of soil and shot from several horizons were ground to pass a 100-mesh screen, dried, and then fused with anhydrous sodium carbonate in the usual way. After solution of the cake with hydrochloric acid and the removal of silica, the determinations of total silica, sesquioxides, calcium, magnesium, and phosphorus were carried out according to official methods. The J. Lawrence Smith fusion of the same samples was employed to obtain data regarding total sodium and potassium. The results of these analyses are presented in table 3.

The data in table 3 indicate that the chemical composition of the shot is different from that of the soil in which they were formed. The silica content of the shot is always lower than that of the corresponding soil. At the same time, the sesquioxide content of the shot is proportionally greater than that of the soil. Associated with this increase in iron and aluminum is a strong enrichment of the shot in phosphorus. From two to five times more phosphorus is contained in the shot than is found in the soil. The enrichment of the shot in sesquioxides indicates also that this material is probably the cementing material holding together the finer components of the shot. The dark color exhibited by a freshly broken shot pellet suggests that iron compounds are chiefly responsible. It is likely that the sesquioxides and phosphates in solution as a result of the weathering of the primary minerals precipitate and then become dehydrated during the dry summer season and, being difficult to redissolve in this condition, they function as permanent cementing material in the shot. The original concentration of quantities of the soluble iron and aluminum compounds around the shot nuclei probably occurs during the early stages in the drying out of the soil. There is not much consistency about the comparative base content in the shot and soil samples, for sometimes the shot and sometimes the soil is richer in calcium, magnesium, sodium, or

potassium. The large variations in relative proportions of the silica and the sesquioxides probably influence the apparent percentage of basic material considerably.

In view of the fact that no definite clay concentration layer and no definite B horizon exists in any of the soil profiles in western Washington that have thus far been analyzed and because of the similarity in the analyses of shot and of B horizons reported for brown and gray forest soils elsewhere, it is reasonable

TABLE 3

*Chemical composition of the shot larger than 2.0 mm. in diameter and of the soil from which the shot were screened*

(Total solution analyses)

SOIL	HORIZON AND SAMPLE	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	Ca	Mg	Na	K	P
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Kitsap silt loam.....	1 shot	56.52	22.62	9.98	1.38	1.62	1.67	1.46	0.439
	1 soil	57.14	20.31	7.98	1.60	1.92	1.53	1.28	0.169
	2 shot	61.45	19.58	7.97	1.60	1.26	1.96	1.40	0.229
	2 soil	65.27	16.81	7.19	1.80	1.74	1.92	1.22	0.047
Kingston loam.....	1 shot	65.97	17.77	5.83	1.75	1.09	1.88	0.96	0.277
	1 soil	75.30	12.57	3.43	1.80	0.87	2.19	1.02	0.128
Clallam sandy loam.....	1 shot	61.75	18.97	7.98	2.05	1.36	1.96	1.01	0.945
	1 soil	69.86	15.16	4.79	2.27	1.14	2.15	1.04	0.250
	2 shot	64.20	17.62	7.58	2.02	1.29	2.01	0.98	0.628
	2 soil	71.15	13.92	5.43	2.32	1.19	2.32	1.04	0.270
Alderwood sandy loam.....	2 shot	58.52	18.52	10.38	2.35	1.69	1.97	1.01	0.479
	2 soil	77.50	9.51	4.79	1.85	0.86	2.09	0.99	0.129
	3 shot	62.65	13.08	9.82	2.45	1.47	2.19	1.03	0.263
	3 soil	73.75	12.87	5.03	1.90	1.00	2.02	0.99	0.128
Wind River loam.....	2 shot	52.35	25.12	8.78	2.40	1.11	1.83	1.24	0.270
	2 soil	58.46	21.03	7.97	2.20	1.09	2.35	1.38	0.118

to look upon the shot pellets in western Washington soils as portions of a diffused B horizon. In place of the usual development of a distinct layer with a reduced silica content and an enriched sesquioxide supply, the B horizon in western Washington soils forms as pellets around suitable nuclei throughout the entire profile. The conditions which cause this unusual development are no doubt the extreme drying out of the soil profile in summer, thus throwing out of solution and dehydrating the soluble iron and aluminum compounds and rendering them relatively insoluble and stable. These materials act as

the cementing agents for the shot aggregates. The more restricted the internal drainage and the less the removal of the soluble iron and aluminum compounds by leaching, the more intense is the shot formation. The shot differ, however, from ortstein because they do not contain so much organic material as is ordinarily associated with this development.

The presence of such a large percentage of phosphorus in the large shot raises an interesting question regarding analytical procedure for the chemical analyses of shot soils. In the case where 60 per cent of the total soil mass is screened out as shot larger than 2.0 mm. in diameter and these shot contain five times more phosphorus than the soil which is left for analysis, it becomes an important question to know whether the phosphorus of the shot is available to plants. If it is, then the arbitrary discarding of a large proportion of the soil, by screening, will result in obtaining entirely misleading data on the phosphorus situation in that soil. Extraction studies with the shot in their natural condition revealed that at pH 2.0 a large amount of phosphorus is readily brought into solution, and, although the surface area of the large shot in the samples was small, strong concentrations of soluble phosphorus were obtained from them in a short extraction period. This indicated that the available phosphorus contained in the shot could not be ignored.

A preliminary experiment with tomatoes growing in quartz sand cultures was then undertaken. A number of containers were filled with quartz sand alone, and duplicate sets were mixed with 10 per cent of the following phosphorus carriers—rock phosphate, ground shot (100 mesh), and whole shot (2.0 mm. and larger). A nutrient solution containing no phosphorus was furnished daily to the plants set in each container, having a phosphorus carrier; and for comparison two jars with quartz sand alone received a complete nutrient solution with phosphorus. The results after 8 weeks' growth are shown in figure 4 of plate 1.

The phosphorus of the shot is evidently available to tomato plants. This and the extraction experiments make it imperative to give consideration to the shot in western Washington soils when studies of available phosphorus are being made.

#### GENERAL DISCUSSION

Chemical analyses of the soils indicate that podzolic soil formation processes have been active in the development of the profiles. This is apparently the dominant process in the forested area on the west coast of Washington. Under the acid condition within the profiles, the iron and aluminum minerals are readily attacked and made soluble. The dry summers, however, inhibit a general downward movement, and no clean cut B horizons are produced. Perhaps because of the abundance of these compounds and their evident activity as cementing substances upon dehydration, their precipitation takes place in part at least around localized nuclei furnished by coarse particles in some cases or by other focal points. The surrounding soil material is fastened

together by this cementing action. Since small shot are encountered in all of the sand fractions down to fine sand, decreasing in proportional amount as the size diminishes, many shot must start other than around a gravel nucleus. In effect the soils of the region have their B horizon scattered throughout the entire profile rather than occurring as a definite horizon. Although individual shot pellets resemble rounded ortstein fragments to some extent, they are too low in organic matter to permit the ortstein designation. They are more similar to the semi-cemented materials of the B horizons of podzolic soils like the Fox series of the central states.

#### SUMMARY

An investigation of the occurrence and of certain physical and chemical characteristics of shot clay soils in western Washington has been made. The results may be briefly summarized as follows:

Shot clays are formed under forest vegetation only and are best developed where there is restricted internal drainage in the profile. They appear to be a natural development in the normal profile of the region.

The proportion of shot in the glacial soils increases with depth in most cases and then decreases again. In the residual soil group most of the shot are found in the upper layer and decrease in numbers with depth (table 1).

The size varies from 0.05 to 19 mm. in diameter in the soils examined. The shot are spheroidal in shape.

Coarse gravel is often found in the center of the larger shot, but the smaller pellets appear to be aggregates of finer soil particles.

The shot often contain more silt and less clay than the surrounding soil matrix, but no striking differences in the percentage of sand separates occur. This might indicate that at the time of cementation the soil was richer in the silt fraction, but, upon further weathering, much of the unconsolidated portion of the soil outside of the pellets has been broken down to the dimensions of clay.

The shot are richer in the sesquioxides and in phosphorus than is the soil surrounding them. On the other hand the soil is richer in silica than are the shot. Phosphorus in particular is concentrated in the shot.

A possible explanation for the formation of these pellets is suggested by the precipitation and dehydration of soluble iron and aluminum compounds around nuclei during the dry summer season. The low solubility of the sesquioxides and phosphates subsequent to dehydration produces a permanent cementing material.

Because of the similarity in composition of B horizons in normal podzolic soils and of shot in western Washington soils, it is reasonable to consider the shot as parts of a diffused B horizon scattered throughout the weathered portion of the soil profile.

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## PLATE 1

FIG. 1. Soil shot from western Washington. The maximum diameter of the largest specimen is 19 mm.

FIG. 2. Typical lumps of shot clay soil, showing the larger shot embedded in the soil matrix. The lump at the left is from the surface soil, the middle one from the subsoil, and the lump at the right is from the clay substratum and contains no shot.

FIG. 3. The appearance of soil shot in a rain washed road cut, showing columns of soil each capped with a shot which protected the soil from being washed away beneath it.

FIG. 4. The appearance of tomato plants after 8 weeks' growth in sand culture, which received the following treatments: *OP*, nutrient solution without phosphorus; *CN*, complete nutrient solution with phosphorus; *RP*, nutrient solution without phosphorus—10 per cent rock phosphate in sand; *GS*, nutrient solution without phosphate—10 per cent ground shot; and *S*, same with 10 per cent whole shot.



FIG. 1

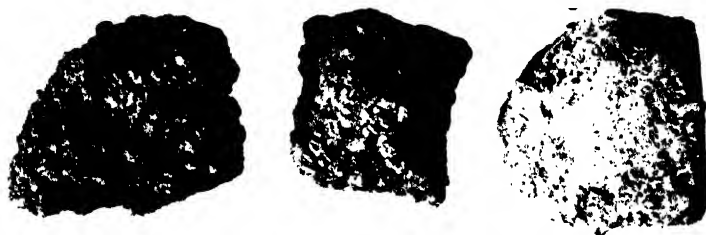


FIG. 2

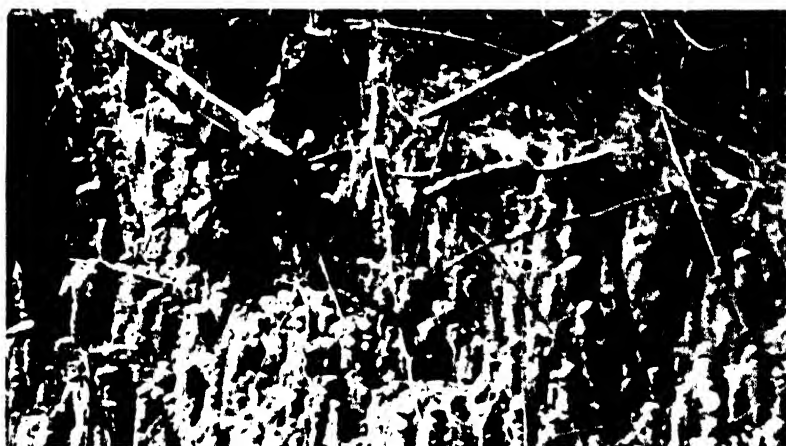


FIG. 3



FIG. 4





# A STUDY OF THE PRACTICABILITY OF THE WALKLEY AND BLACK METHOD FOR DETERMINING SOIL ORGANIC MATTER<sup>1</sup>

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The need for a rapid approximate method for organic carbon determinations in soils which can be depended upon to give comparable results has long been recognized, and several investigators (3, 4, 5) have endeavored to fill this need. The recently published work of Walkley and Black (7) gives promise of more nearly meeting the requirements of such a method than any previous one. It was considered of interest, therefore, to investigate the practicability of the Walkley and Black method by comparing it with the wet combustion method in which are used various soil types to which known quantities of organic matter have been added in the form of straw.

The seven soil types used in this work were selected for their wide differences in origin, physical characteristics, organic matter content, and location. These are listed in table 1.

Gravel and foreign material were thrown out, and the soils were sieved through a 60-mesh screen and oven-dried at 105°C. for 12 hours. Each soil was divided into three portions, two portions being weighed and thoroughly mixed with 1 and 2 per cent, respectively, of finely ground straw which had also been sieved through a 60-mesh screen. The total carbon contents of these samples were run by the Walkley and Black method (7) and also by the A. O. A. C. wet combustion method (2). Since various investigators (1, 8) have found that the dry combustion and the wet combustion methods give similar results, the latter was selected because of its convenience.

The carbon content of the straw was 43.5 per cent as determined by the wet combustion method. No attempt was made to determine the carbon in the straw by the new method because the high carbon content of this material necessitates the use of such exceedingly small samples that accurate results are difficult to obtain.

The carbon contents of the seven soil types as obtained by the two methods are given in table 2.

The results obtained by the two methods agree very well for the Melbourne,

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Palouse, and Everett soils. The Chehalis, Puget, and Spanaway soils gave high results by the new method. The results for the Ephrata soil were satisfactory for the check soil but much too high when straw was added.

TABLE 1  
*Description of the soil types used*

SOIL SERIES	CLASS	LOCATION IN WASHINGTON	PARENT MATERIAL	ORGANIC MATTER*
				<i>per cent</i>
Spanaway	Sandy loam	Central western	Glacial, outwash plain	4.08
Everett	Gravelly sandy loam	Northwest	Glacial till	4.49
Puget	Silt loam	Northwest	Alluvial, outwash of glacial	3.56
Melbourne	Silty clay loam	Southwestern	Residual	5.06
Chehalis	Silty clay loam	Southwestern	Alluvial, outwash of residual	4.62
Ephrata	Very fine sandy loam	Central	Alluvial, river terraces	0.49
Palouse	Silt loam	Southeastern	Loessial	2.50

\* As determined by A. O. A. C. wet combustion method.

TABLE 2  
*Comparison of the organic carbon contents of seven soil types as determined by the wet combustion and the Walkley and Black methods*

SOILS	CHECK				1 PER CENT STRAW ADDED				2 PER CENT STRAW ADDED			
	Wet combustion	Walkley and Black C X 1.32	Walkley and Black*	Recovery†	Wet combustion	Walkley and Black C X 1.32	Walkley and Black*	Recovery†	Wet combustion	Walkley and Black C X 1.32	Walkley and Black*	Recovery†
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Spanaway sandy loam.....	4.08	4.38	3.29	80.6	4.41	4.75	3.56	80.7	4.76	5.17	3.88	81.5
Everett gravelly sandy loam..	4.49	4.56	3.42	76.2	4.87	4.99	3.74	76.8	5.22	5.44	4.08	78.2
Puget silt loam.....	3.56	3.97	2.98	83.7	3.98	4.52	3.39	85.2	4.34	4.85	3.64	83.9
Melbourne silty clay loam....	5.06	5.08	3.81	75.3	5.50	5.55	4.16	75.6	5.86	5.93	4.45	75.9
Chehalis silty clay loam.....	4.62	4.85	3.64	78.8	5.03	5.35	4.01	79.7	5.44	5.68	4.26	78.3
Ephrata very fine sandy loam.	0.49	0.49	0.37	75.5	0.93	1.02	0.77	82.8	1.27	1.53	1.15	90.6
Palouse silt loam.....	2.50	2.33	1.75	70.0	2.90	2.82	2.11	72.8	3.32	3.34	2.51	75.6

\* Conversion factor, 1.32, omitted.

† By Walkley and Black method (conversion factor omitted) based on results of wet combustion method.

Because of the consistency of the approximate method in giving nearly parallel but slightly higher results than the wet combustion method it appears that the former method accounted for nearly all the straw added. This is

indicated also in table 2, which shows the percentage of carbon recovered by the new method, the wet combustion method being used as the standard and the correction factor proposed by the authors of the new method being omitted.

It is apparent that soil type influences the percentage recovery of the total carbon present, but for western Washington soils, for any one type with its varying organic matter additions, the percentage recovery is approximately the same. The central and eastern Washington soils, however, are not so consistent.

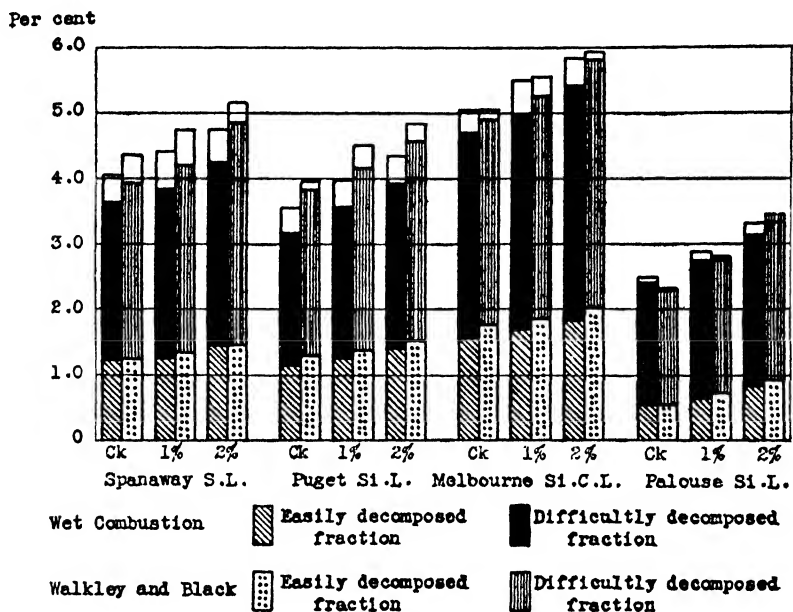


FIG. 1. THE EASILY AND DIFFICULTY DECOMPOSABLE FRACTIONS OF SOIL ORGANIC MATTER, IN PER CENT CARBON, AS DETERMINED BY THE WALKLEY AND BLACK AND THE WET COMBUSTION METHODS ON FOUR SOILS, EACH UNTREATED AND TREATED WITH 1 PER CENT AND 2 PER CENT STRAW AS INDICATED

Unshaded portions represent difference between the sum of the fractions and the total in original soil.

Since Walkley and Black (7) found an average of 76 per cent recovery of the total carbon in the soil by their method, they assumed that there is a definite fraction of organic matter common to many soils which is readily attacked by oxidizing agents. By removing the simpler organic fractions from the soil and separately determining the carbon in the soil residue and in the organic fraction removed, it might be possible to determine in which fraction the disagreement of the two methods lies. The soils were treated with 80 per cent sulfuric acid, as worked out by Waksman and Stevens (6). This treatment removed the starches, hemicelluloses, and celluloses by filtration and washing with hot water. Lignin and lignin-like materials were left in the soil. The

filtrate was reduced to a definite volume, and aliquots were used for determining the total carbon content. Total carbon was also determined on the treated soil residue. The results for four soils are graphically represented in figure 1.

Of the four soils studied, three—Spanaway, Puget, and Melbourne—were from western Washington; and the other, Palouse, was from southeastern Washington. The two methods agree fairly closely for the simpler organic fraction; i.e., starches, hemicellulose, and cellulose extracted by the use of 80 per cent sulfuric acid. Since the agreement is so close, it may be assumed that it is not these fractions which give the observed differences between the two methods for the original soils.

When the results of the two methods are compared for the more resistant fraction of the organic matter they are shown to be in about the same ratio as in the unfractionated soil. This would seem to indicate that the major differences in the results by the two methods originate in the more resistant fraction of the organic matter. It is also possible that some of the observed differences may be due to the presence of inorganic oxidizing or reducing substances as suggested by Walkley and Black (7).

TABLE 3  
*Effect of alkali carbonates on method*

	OFFICIAL			WALKLEY AND BLACK
	Total C	CO <sub>2</sub> C	Organic carbon	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Soil No. 1.....	1.728	0.534	1.194	1.32
Soil No. 1 + Na <sub>2</sub> CO <sub>3</sub> .....	2.470	1.282	1.188	1.31
Soil No. 2.....	0.444	0.099	0.344	0.40

#### EFFECT OF CARBONATES IN THE SOIL ON THE WALKLEY AND BLACK METHOD

Since this method is not dependent on CO<sub>2</sub> evolution no correction is necessary for inorganic carbonates in the soil, and, therefore, the method should be well adapted for determining the approximate organic matter content of soils containing carbonates. The data presented in table 3 are averages of closely agreeing duplicate determinations, and although they do not cover a wide range of soils the results bear out this assumption. The relationship of the results obtained by the two methods on these soils is very similar to that of the results of the other seven soils considered in this study. Thus this method may have particular merit for use with soils containing carbonates where time is a factor and absolute accuracy is not essential.

#### SUMMARY

A comparison was made of the Walkley and Black approximate method with the wet combustion method for the determination of organic carbon in seven widely different soils, portions of which received 1 and 2 per cent of straw

respectively. The organic matter in four of these soils was fractionated by extraction with 80 per cent sulfuric acid, and the organic carbon in the two fractions was determined separately by both methods. The effect of carbonates on the new method was also briefly considered.

The results obtained by the approximate method are slightly higher than those obtained by the wet combustion method but in general they are parallel. The approximate method accounts for practically all of the organic matter that was added in the form of straw.

When the organic matter was divided into the easily and difficultly decomposed fractions the results of both methods agreed very well for the former, but the same general differences observed for the results on the unfractionated soil were found in those for the difficultly decomposed portion of the organic matter. Probably the more resistant forms of organic matter and possibly certain oxidizing or reducing substances present in the soil are responsible for the higher results obtained by the approximate method.

The Walkley and Black method is adapted for determining the approximate amount of organic matter in soils containing inorganic carbonates without correcting for their presence.

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# DIRECT MICROSCOPIC AND BACTERIOLOGICAL EXAMINATION OF THE SOIL

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ASSISTED BY

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The object of this paper is to summarize in English the methods previously published by the writer (3-12) in Italian and German and to explain the most important results obtained with these methods.

## METHODS ADOPTED IN OBSERVATIONS

### *Preparation of the soil sample by impression*

Although the impression method is now somewhat out of favor as compared with the preparation of the soil sample by crushing, to be described later, it seems advisable to refer to it, as it is still of certain value in many cases. In putting it into practice a kind of soil impression apparatus is employed. This is essentially a device to hold a microscope slide in such a manner that it can be pressed against the surface of soil. At first the writer smeared this slide with a solution of agar or gelatin, but the present technic uses a clean slide thoroughly sterilized by being passed 50 times over a flame or, when considered necessary, also treated with distilled water.

The material remaining attached to the slide is then fixed by heat in the usual way, stained by the Conn method with carbol erythrosin (1 per cent erythrosin solution with 5 per cent phenol) employing heat, and thoroughly washed and dried.

In the case of a heavy soil, this slide is pressed against a fresh cut made with some sharp instrument. With a sandy or crumbly soil it is necessary, however, to prevent any lateral slipping. To this end metal sheets may be used with a sharp cutting edge and forcibly driven into the soil with a mallet, in this way marking out on three sides a parallelepiped of ground. Next, with another squared sheet corresponding in width to the median of the three sheets previously driven into the ground, the soil immediately above the layer which is to be sampled is lifted off, naturally after removal of the soil in front so as to ensure free space for the operation.

The soil impression may be taken parallel to the surface or obliquely or against a perpendicular face; the results do not vary greatly especially if the impression is taken at a certain depth. Impressions made at the surface are not satisfactory.



*Preparation by crushing*

For the crushing method a small lump of soil is crushed on the surface of a microscope slide. The crushing takes place without artificial means when the soil is sufficiently soft; otherwise, the selected fragment is placed on a glass or a porcelain slab and then sprinkled with as much distilled water as it is considered capable of absorbing; for this a few minutes will suffice. It will then be easy to obtain preparations by impression (Klatsch Preparat).

In many cases the crushing can be done between a pair of slides without using the apparatus. The drying process can be carried out before separating the two slides, and thus two prepared slides can be obtained simultaneously.

The microscopic examination in all cases is made with the objective in immersion, the preparation being covered with a slip or the oil being run directly on the preparation.

*Observation of the lower face of the preparation*

It is often desirable to study the lower side of a preparation as well as the upper side. In doing this a cover slip is prepared and stained by the method described hereinafter for counting cluster forms (golumerles). After the cover slip has been fixed with cedar oil on a slide with the oil between the upper face of the slide and the lower soil-free face of the cover slip, it is then examined with an objective immersed in another drop of cedar oil on the soil side of the cover slip. An easily recognizable field is then found and carefully studied, after which the cover slip is detached from the slide and replaced with the other side up, *rotating about one of its sides which is parallel with the longitudinal side of the slide*. One then has to locate the same field examined with the other side up. This is sometimes difficult but can usually be done with the assistance of marks made on the stained cover slip with a needle dipped in a violet dye. Especially valuable in relocating the field is a rough sketch of the field in question drawn on transparent paper which can be turned upside down to show the appearance of the desired field when it is to be studied in its second position.

*Method of counting the cluster forms*

The count of the groupings or accumulations reveals cluster forms far more frequently, as we shall see. To this end, from a very summary method based on the number of clusters present in 16 fields of the prepared slide, we have, with the aid of an expert mathematician,<sup>1</sup> developed one far more accurate which is carried out in the following way:

A number of ordinary glass cover slips are weighed and, to keep them distinct from one another, are placed on numbered squares on a sheet of paper. Each slip is then covered with small lumps or fragments of soil which are moistened to their full absorption capacity. Crushing is then accomplished

<sup>1</sup> Giovanni Candura.

with a slide or by means of the soil impression apparatus. After drying, the cover slips are stained in 1 per cent erythrosin dissolved in 5 per cent phenol, heated to steaming over a flame, washed in cold water, and dried over the flame. Each cover slip is then weighed again, the difference between this weight and the previous one giving the weight of the attached soil. The cover glass is then attached to a slide and examined under oil immersion. The clusters present in 20 fields are counted.

The Candura formula (5) is then applied, that is: taking  $P$  as the weight of the soil in grams,  $S$  as the area of the soil sample in square centimeters (*i.e.*, the area of the whole coverslip holding the soil sample),  $s$  as the ratio  $\frac{S}{P}$  (specific area of the soil),  $a$  as the number (*viz.*, 20, as already stated) of the fields observed,  $N$  as the sum<sup>2</sup> comprehensive of the clusters seen in  $a$  fields,  $n$  the ratio  $\frac{N}{a \pi r^2}$ , that is the number of clusters per centimeter, we shall obtain:

$$n s = A$$

that is to say,  $A$  will be the number of the cluster forms per gram of soil.

In employing this method it is convenient to use two, four, or eight (usually four) series of 15 cover slips each and to apply the Candura formula to each slide. The fewer the cluster forms in the preparation, the larger the number of series will be needed. The object is to make a sufficient number of determinations so that the figure obtained upon averaging the results from half the cover slips will be approximately equal to that of the other half. Further details and notes of difficulties surmounted may be found elsewhere (12).

#### METHOD ADOPTED FOR OBTAINING MATERIAL

At present one method only is in use, *viz.*, *burial of the slides*, which has been employed by the writer (1, 4, 5, 7, 13) since about 1927.

Pairs of slides may be buried at a greater or less depth and extracted after a given time; they are then stained in the same way as soil samples prepared by crushing. Experiments have shown that the soil bacteria pass over the slide both on the upper and on the lower face, and not only the schizomycetes but also the hyphomycetes, streptothrices, and protozoa.

It is necessary to use pairs of slides because one of the two faces—the lower face in the case of the upper slide and the upper face of the lower slide—must be put out of action by the necessity for staining and observation. In other words, the lower face of the lower slide acts as the lower face of the upper slide.

<sup>2</sup> The authors take this opportunity of pointing out that on p. 238 of our article (12) the value of  $N$  is erroneously given as that of the *area occupied by 1 gram of soil* instead of, as on p. 15 of the article (5) which, however, must be corrected definitively: *the sum comprehensive of the clusters seen in a fields*.

At the moment of burial the slides are passed through the flame 50 times in order to purify them of any substance which might have chemotactic action on the soil bacteria, *unless it is preferred to smear them with substances of which it is desired to study the possible chemotactic capacity.*

In dealing with natural soil *in situ*, and if the earth is compact and can be sliced, the apparatus we have described for taking soil impressions at a depth will serve very well. Having made the cut at the required depth, the pairs of slides are placed in position and covered as far as possible with the slices of soil taken from the cut, which are pressed gently into place. The opening made in front of the cut is also filled in, if possible replacing the earth in the same order in which it was removed. Particularly in dealing with grassy turf it is advisable to keep the surface sods intact, putting them back into position over all the disturbed area. It is not advisable to water in this case.

#### METHODS OF REPRESENTATION AND DESCRIPTION

One of the greatest difficulties in these studies is to convey what we have seen to other students who do not wish to verify our observations by repeating them. Illustrations have been given in color in previous articles, but are not reproduced here because of the expense. For the benefit of those who are interested in studying them and who have, or can obtain, the preceding articles, the eight plates previously published are referred to here (in Roman numerals) according to the following scheme:

Plate I (9, plate I); Plate II (9, plate II); Plate III (10, plate VI, and 5, plate I); Plate IV (10, plate VII); Plate V (3); Plate VI (11); Plate VII (8); Plate VIII (13).<sup>3</sup>

As to the method of reproduction, we naturally had not much liberty of choice between a drawing from the microscope and microphotography. Plates I to IV are examples of the drawings; and Plate V, fig. 10, 11, 12, and Plate VII, fig. 49 *a, b, c*, 50, and 51 are examples of the microphotography. The latter have been rendered more explanatory by coloring. These drawings are not claimed to be absolutely accurate reproductions because of three sources of error: first, the fact that the draftsman is not always scientifically trained; second, that the reproduction of color with artists' paints is not always exact; and third, that their reproduction in printing by the ordinary three-color plate method is still more difficult.

In the preparation of these drawings we have preferred a magnification of 750 diameters rather than one of 2,000 diameters as employed by Winogradsky.

Morphological observations made over a period of approximately 8 years on the groupings of schizomycetes and other microorganisms visible in the soil can be summed up in the following way, and the groupings themselves may be classified morphologically as will be shown later. It must, however, be premised that by the term *colonies* we mean something which has practically nothing in common with those which we are accustomed to find in agar gelatine

<sup>3</sup> *Sup. Proc. Internat. Soc. Soil Sci.*

cultures, particularly because they are very much smaller and barely reach the size limits of the deep-seated colonies therein immediately after development.

*Filmy colonies.* These mainly consist of elongated schizomycetes (bacteria or bacilli), varying in length between 1.5 and  $4.2\mu$  and in breadth between 0.4 and  $1.00\mu$  (Plates: I, fig. 6; II, fig. 1; III, fig. 1 and 4; V, fig. 6; VII, fig. 45, 46, 47, 48<sub>1</sub>, 48<sub>2</sub>, 48<sub>3</sub>). Although for the most part microscopic, colonies are often found as large as the field of the microscope itself (eg., diameter  $220\mu$ ); hence, when complete and intact, they correspond to, or approximate, the size limits of the colonies deep-seated in agar cultures.

They all resemble the bacterial *films* of varying consistency, sometimes cartilaginous, which are formed on the surface of nutritive liquids. They too can be folded back—possibly by the action of the apparatus or generally of the manipulations—and the edges may be superimposed.

If an intercellular substance exists, it does not take the *erythrosin staining*. These colonies are sometimes visible to the naked eye, as may be seen in figure 10 of Plate II, on which is clearly shown the folding back, at times more than one fold apparently being present. There exist forms which may be called "filmy colonies," which are not clearly disclosed by the microscope (ultra-microscopic colonies?) (Plate I, fig. 3). And still other forms exist resembling folded scraps, as though developed within the irregular openings between the mineral particles into which they fit.

*Schizomycetic masses in cluster form.* This is by far the most frequent form and the one we regard as characteristic of the soil in which it is found. These are the most frequent apparent types of the schizomycetes in the soil, and we have here an instance of groupings, which by their nature can never be of a large number of individuals (essentially of coccic or coccobacteric forms), because, in spite of many thousand observations, these groupings have *never* been found to assume any considerable proportions, except in the case of the *giant clusters* of which mention is made in another report (13) (Plate VIII, fig. 2 and 3). The number of constituent forms, so far as it is possible to count them, hardly exceeds 30; only when they assume the appearance resembling the embryological *morula*, forming compact masses, the number of individuals must be some hundreds. One of the largest areas is also reproduced in Plate V, fig. 3. It is not difficult to observe aspects which may very well be interpreted as points of transition from forms with a small number of individuals, and even of isolated individuals, to the *morula* form.

Now if 30 may be considered as the number representing the average maximum of microorganisms composing these groups, which from now on we will call *clusters*, or *glomerules*, the minimum number varies greatly and may even be supposed to be two only. There are fields in which the groupings are numerous with a varying number of components, but where there are also present isolated individuals (e.g., Plate III, fig. 11). In this case one hesitates to assume that it is either a question of new clusters being formed, as it were, from parent clusters, or one merely of remains of larger clusters broken by the soil impression apparatus or by heat or by washing. It is difficult to admit that we have here merely remains of larger colonies, since as already stated *no colonies so large have ever been found*.

But in support of the view that the clusters have a morphological individuality formed only of few individuals as mentioned, there is the fact that in the large majority of cases, the *cluster* is not independent, as if it were superficially attached to a mineral fragment, but it is at the center of something which encloses it, as shown also in the figures of Winogradsky (14). The cluster, in fact, seems as though enmeshed in a membrane of plastic material which has been crushed under the action of the apparatus, opening the "envelope" and revealing the schizomycetic grouping, as shown in figures 6, 7, 8, 9, 10 of Plate III, less well marked on figures 1 and 2 of Plate V, and very well marked on figures 30 to 37 of Plate VII. In these last the appearance is exactly that of the schizomycetic cluster escaped from an envelope broken under the pressure of the apparatus. But even where there is no gap between schiz-

omycetes and the enclosing substance (and it is impossible to tell whether this space which appears to us to be void is really filled with material, which in contradistinction to the casing is *not stained*), it is clearly seen in figures 1 to 42 of Plate VII that all the clusters are immersed in a soft, and one may say, plastic substance. This substance appears to be differently stained from the schizomycetes, with that intensity of contrast which we are accustomed to note, e.g., in the twofold staining of the acidophils or in histo-cytological stainings, and according to a chromatic scale which excludes only the colors of the most highly refracting part of the spectrum.

In counting the clusters of a field under examination, we have always conformed to the rule of neglecting the isolated forms, or those linked in twos or threes only, or even more, when in the same field there were also present more numerous groupings, or when the groupings reduced to a scanty number of individuals were assembled in "nests" not differing from those composed of numerous individuals.

As regards the form, there can be no doubt that the "coccic" is the prevalent form, but the *possibility must always be kept in view* that we may have, instead, cocco-bacteria, which are frequently found in conjunction with pseudo-cocci. A very variable characteristic, however, is the size even in the same preparation, as typically seen in Plate V, fig. 2.

There may rarely be found together (Plate III, fig. 7) cocci "nested" together, bacteria and bacilli with non-typical forms, and still more rarely (Plate III, fig. 6) a few individuals clearly of bacillar type "nested" like the cocci.

All these aspects occur if a thorough examination is made of the colored figures of Winogradsky.

*Streptothrices.* In contradistinction to Winogradsky, we have been able to discover these in the typical hyphic and stromatic forms (Plate II, fig. 3, Plate IV, fig. 6, and Plate V, fig. 12) and also to prove their presence by the use of the buried slides method.

*Hyphomycetes.* These we have found very frequently. An example is given on Plate II, fig. 3, and Plate VI, fig. 8. It frequently seemed quite easy to distinguish whether the forms under observation were dead or still living forms.

*Other forms of schizomycetic colonies.* These are quite rare, and we think (especially when dealing with soils that are rich in decomposing vegetable matter) that such of them as might be interpreted as special forms (e.g., Plate II, fig. 2, and Plate V, fig. 9) are only filmy forms developed in special conditions, whether bacteric or bacillic, according to the distinction already made.

A particular form (which there is reason to believe is due to the same type of colonies and their components) is that made up of individuals with every probability and appearance of being spore-forming (Plate II, fig. 11, Plate III, fig. 1*b* and fig. 3, and Plate VI, fig. 2).

Very occasionally, groups have been found which appear to derive from the *remains of colonies* broken up by the soil impression apparatus, and made up of individuals suggesting the Amylobacters (Plate V, fig. 8) as not showing stain at the two extremities.

*Protozoa.* Protozoa in the form of cysts are found isolated, but, on the other hand, free protozoa (flagellate but also other forms) are found comparatively frequently grouped in masses which only the lack of accurate knowledge makes us hesitate to call "colonics" (Plate I, fig. 2; Plate II, fig. 5 and 12; Plate III, fig. 2; Plate V, fig. 11; Plate VI, fig. 1).

We have also, in all probability, observed casings of radiolar and foraminiferous forms in soils of undoubted marine origin.

*Isolated forms.* To what we have already said elsewhere on the subject, we can only add that, in soil strata other than the superficial layers, compact little "crowns" of *streptococci*, or possibly streptothrices, are often observed. Chains of *streptobacteria* are more rarely discernible.

We do not deny, however, that many other almost or entirely isolated forms may be found in the soil—in fact, they are frequent; but such observations as we feel justified in recording are the following:

1. The only formations which are numerous and are constantly present under the condi-

tions already specified and under those to which we shall later refer, are the filmy colonies and the clusters.

2. *All the others*, including the streptothrices, hyphomycetes, diatoms and protozoa, may be found sporadically and in some preparations in considerable numbers. These do not, however, form in the corresponding soil anything that can justify the view that they are an important or constant factor in determining its structure.

3. None of the isolated forms in small groups (such as the "crowns" of streptococci and thus coccic, bacteric, and bacillic forms) apart from the already described filmy and clustered colonies, take the form of *colonies* even as small as the filmy and clustered kinds.

*Algae.* Although we cannot deny the possibility that various unicellular algae may be found in the soil, we have identified numerous species, both on the surface and at comparatively deep levels, which undoubtedly suggest only the diatoms.

#### SIGNIFICANCE AND IMPORTANCE OF THE FILMY COLONIES

The observations made can be classified as follows:

1. Impressions of topsoils or of soils immediately below the layer of dead leaves, where this existed.
2. Impression made by raising the layers of moss or of lichens found either on the ground or on walls or trees, thus laying bare the layer immediately beneath.
3. Removal of plants from loose or soft soil, keeping the roots intact so that a small amount of soil is brought away with them, and an impression then taken of the larger among the small soil lumps thus obtained.
4. Excavation of the soil *in situ* and impression (or removal of small lumps) at depths of 5 cm., 10 cm., 15 cm., etc.
5. Making up of pots of soil mixtures, Vesuvian sand, and leaf mould, whether sieved or otherwise, in varying proportions. Examination after varying periods and in various conditions of preservation, particularly with regard to the water content.

By direct observation we have discovered the following facts:

1. By *impressing* superficial soil when the ground is damp and the plants fresh, we may note many bacilli all similar in type to those of the filmy colonies, and sometimes even filmy colonies themselves.
2. A similar result is given by lifting the layers of moss, hepaticas, and lichens in damp, shaded places and impressing the underlying mould.
3. The filmy colonies are in direct proportion to the quantity of organic matter in a state of decomposition in the soil under examination, to the weather, and to the temperature at which the soil mixture was prepared.

Giant filmy colonies found in pots holding a large proportion of leaf mould are reproduced in Plate VII, fig. 45, 46, 47, 48<sub>1</sub>, 48<sub>2</sub>, 48<sub>3</sub>.

#### SIGNIFICANCE AND IMPORTANCE OF THE CLUSTERED COLONIES

##### *Observational research*

With the methods described and with all proper safe-guards, we have carried out two series of tests, one by observation and the other by experiment. The principal results obtained by simple microscopic observation and by counting the clusters are as follows:

*Number and distribution of clusters in Italy.* The smallest number of clus-

ters observed per gram of soil was 22,032 at Parco Gussone (Portici); the greatest, 13,416,699 at Benevente and 13,548,000 at Maccarese.

In both cultivated and natural soils the number of clusters tends to diminish with depth.

In ordinary arable strata (from 0 to 30 cm.) the numerical variations found in depth are of the same order as the numerical variations in extension.

Similarly in depth a given average of clusters generally holds good for strata of approximately 30 cm. thick.

In our experiments we have found the quantity of clusters to diminish from 10 to 20 million to a few thousand in descending from the surface to a depth of 1 meter.

*Geographical distribution of the clusters.* Winogradsky has already demonstrated, as the result of his research work from 1926 onward, that the microflora of the regions of the globe situated in the five continents does not vary greatly qualitatively, at least under direct microscopic examination. He did not, however, consider the matter of numbers, nor did Conn or Cholodny. It is our opinion that all previous research workers concerned themselves only with looking for *given forms* (the typical zoogloea) and must have tested soils from analogous strata, as the forms we have found and described prove that those of Winogradsky, Conn, Cholodny, et al. are not the only forms which are found and which may develop in the soil.

It may be added that in soil from the neighborhood of Hong-Kong we have found the same forms as in soils from other parts of the world and also in similar numerical series.

*The soil clusters in relation to soil cultivation and the duration of cultivation.* There is a remarkably clear relation between the *agricultural age of the soil* (by which is meant the number of years from which its human exploitation probably dates) and *the number of clusters*. The older the soil, the greater is the number of clusters; thus very few are found in soils the cultivable strata of which are only 28 years old, as at Parco Gussone (at Portici). For example, we find that soil (from Maccarese) which has a history of cultivation dating back some 3,000 years, gives 13,548,000 clusters per gram, whereas virgin soil (from Rive Pelate di S.Polo d'Enza) gives 412,119, and soil erupted from Vesuvius in 1906 has, to date, accumulated 40,392 clusters only.

The soil of a vegetable garden—one of those Neapolitan gardens which, with the aid of water, manuring, cleaning, and sun, gives five and six crops of superb products a year—was covered by Vesuvian ash in 1906 to a depth of about 25 cm. This ash was turned in, and as a result there was an increase in the number of clusters but so small as to be almost negligible when the numbers which are known to occur in rich soils are considered. This may indicate that the formation of clusters is a very slow process and *has no relation to the immediate productive capacity of the soil*.

*Experimental research*

*Effects of physical agents on the cluster forms.* The earlier research work consisted simply in placing the soils in which the clusters had been under examination, in a bacteriological thermostat for a longer or shorter period for the purpose of re-examination. It is then seen that a rise in temperature has an influence on the turgidity of the clusters and renders their staining brighter even in the space of only 40 hours.

Subsequently we had occasion to test two soil samples which had been left undisturbed in saucers containing a little water, renewed when evaporation seemed imminent. The saucers were covered with glass slips, but the closing was by no means hermetical; consequently, conditions remained undoubtedly aerobic all the time.

Examination of these soils after 7 years showed that under these conditions of humidity the clusters still remained, and one was observed (Plate VIII, figs. 2 and 3) which could only be defined as a giant type, so large that to sketch it two representations were necessary showing different focal levels.

This, together with apparent fragments of broken-up clusters observed suggested that the humidity had, so to speak, set up germination of at least part of the clusters, that is to say, a multiplication of individuals. Some of these individuals had subsequently been unable to carry out their normal chemical processes and had degenerated and disappeared. To investigate this point, direct experiments were carried on to note the action of water on the cluster forms under four distinct conditions of humidity as follows:

1. *Humidity at air saturation point* for which the soil samples were kept in closed containers with water on the floor of the container.
2. *Humidity at saturation point of the soil.* This result is obtained in practice by immersing the soil fragment in water, subsequently removing it, allowing it to drain, and then keeping it in a container such as the one previously described.
3. *Continuous immersion of the soil sample in water*, under the same conditions as the foregoing ones, with special precautions to prevent evaporation of the water.
4. *Natural humidity*, used as a control.

The results, which agreed fairly well in the majority of instances, showed that *humidity* in three cases gave a larger number of clusters (or a greater visibility due to the greater turgidity, according to our first observations?), but imbibition in three cases quickly reduced their number and with some delay in the fourth case, whereas the immersion process concluded by reducing very considerably the number in all cases.

*Longevity and the preservation of the cluster forms in agricultural soil.* It had already been known<sup>4</sup> for some time that the bacteria, including the non-

<sup>4</sup> See, *inter alia*, the enquiry of GILTNER, WARD, and LANGWORTHY, H. VIRGINIA 1916 Some factors influencing the longevity of soil micro-organisms subjected to desiccation, with special reference to the soil solution. *Jour. Agr. Res.* 5: 927-942, Abs. in *Centbl. Bakt.* (II) 49: 469 (1920). These writers attribute this phenomenon to the capacity of the soil for retaining moisture in hygroscopic form in as much as there seems to be no proportionate relation between the two phenomena. The prevailing clayey nature of the soil, on the other hand, would have an effect on the phenomenon.



sporing types, preserved their vitality for a long time in soil samples kept in the air.

For our own part, from 1930 onward, we ascertained that cluster forms readily taking erythrosin stain can be observed in soil samples of different degrees of age and preservation left on the shelves of the laboratory without any special attention.

In the tables given in the various reports, serial numbers are used to indicate the different samples which we have so far been able to observe. These have been proved to retain cluster forms even after 38 years of preservation in simple glass jars and even in open cardboard containers.

The number of the cluster forms for 50 microscopic fields varied from a minimum of 2 at 20 years of age to a maximum of 149 at 19 years of age, naturally in soils markedly different.

*Action of agricultural operations on a small scale.* An investigation was made of the effect of different methods of cultivation. The plots in this experiment were:

1. A plot lying fallow, which naturally acted as control;
2. A plot worked with a plough;
3. A plot worked by drilling implements (Marienburg drill);
4. A plot worked with various implements.

In other respects the plots remained alike for nearly 8 years.

The results of this experiment were all negative, none of the plots showing variations great enough to be regarded as of any significance.

*Experiments with buried slides.* In various contributions (3, 4, 10, 13) we have given accounts of a number of experiments carried out by the method described elsewhere in this report.

The results may be summarized as follows:

On the buried slides, in soils of all kinds, both bacterial clusters and filmy colonies may be found, especially on the lower face. Much more frequently are found the schizomycetes, both in isolation and grouped in ill-defined masses, hyphomycetes, streptothrices, and perhaps also desmobacteria and protozoa. There are also microorganisms that are difficult to classify, possibly including the mycobacteria of Krassilnikow (2a).

On smearing the slides with various solutions corresponding to fertilizing substances (such as sulfate of ammonia, asparagin, peptone, mannite, rice starch) in concentrations corresponding to such as would occur if used on the soil, no chemotactic effects were to be noted, apart from the influence of the starch, which brought about an abundant increase in the streptothrices and hyphomycetes of various forms, such as have been described in full detail in the articles already mentioned.

Certain groupings of hypho- and schizomycetes are clearly characteristic; the former seem to be lifeless and in process of destruction by the schizomycetes (Plate VIII, fig. 5).

There often appear on the slides what may be called "drop-forms" (sgocciolature). They are apparently produced by drops of moisture charged with bacteria coming in contact with either the upper or the lower surface of the slide and subsequently drying and leaving the bacteria attached to the glass.

The characteristic of these drop-forms is that they have not the least appearance of "*colonies*." In a few very rare instances and only to a very small

extent, they suggest the filmy colonies described elsewhere, but in most cases they consist of schizomycetes of various kinds irregularly distributed, the prevalent forms being bacteria and bacilli, but also including *cocci*, which in the majority of cases are characteristically *very small indeed*. Sometimes (Plate VIII, fig. 8) a considerable doubt remains as to the category of organisms to which they should be properly assigned.

*The so-called colloids and their behavior in regard to anilin staining.* Winoogradsky has called attention to the masses, probably of colloidal nature, which also stain with erythrosin in the slides stained to show bacteria and other microorganisms. We found such material to stain much less uniformly than the microorganisms, and in Plates II, V, and VII, we have shown it as staining red, red-yellow, yellowish, or yellow-gray. We have concluded that the shades of color seen in such material were not caused by the erythrosin but were either natural or the result of heating in the course of preparation. In support of this view we have described a certain number of slides contrasted with other unstained slides of the same soil and also of similar preparations treated under heat with a 5 per cent solution of phenol (as in the case of the Conn staining formula) but without any dye.

The colors are fairly uniform with each soil, fertilizers having no modifying effect.

*Relations between the clusters and the absorbing root hairs.* By various methods it was found possible to obtain fragments of roots and root hairs in the same preparation with the soil and the microorganisms therein. Such experiments showed no static or numerical relations between the clusters and the absorption apparatus of the plants, but the clusters were concentrated about the roots. The preparations made under natural or artificial conditions did not differ greatly from normal preparations in which roots, root hairs, and rhizoids had been intentionally or unintentionally introduced; they might or might not contain cluster forms like any other preparation; and, like any other preparation, they contained or did not contain filmy colonies, scattered bacteria, etc.

This was the first fact that led us to conclude that the cluster forms indicate a state of repose and not a trophic state on the part of the soil schizomycetes.

*Specific identification of the flora observed under the microscope.* An experiment has been carried out on the basis of which, after repetition and confirmation by other experiments, we should at least be in a position to deny to the cluster forms the character of *Azotobacters*.

From a seedbed containing sandy soil in which wheat grains have been placed to germinate, a seedling is uprooted, one of the branches of the radicle is deprived of the apical part, and the portion with abundant absorbent root hairs is divided into two parts.

One part is placed on an object glass, and water is added without crushing the plant material; it is left for a few minutes and then dried by means of a fan.

There is a considerable deposit of material which, observed under the microscope, contains:

Scattered *cluster forms*, some reduced and others typical and very pronounced.

Groups of *cocci* in chain form.

Small groupings or accumulations of delicate bacilli.

A very large number of tiny delicate bacilli, irregularly arranged, but observed only in a few fields. In all probability they develop from pre-existing colonies as a result of the water used in making the preparation.

The second portion is placed with all the soil attached in a test tube containing 10 cc. of distilled water, and well shaken. With the Hiltner and Störmer method (2b) and the processes described elsewhere (2), it is possible to determine the quantity of Amylo- and Azotobacters, and of ammonifying and denitrifying organisms, contained in the liquid derived from the suspension obtained.

The following results were noted:

Amylobacters.....	none
Azotobacters.....	none
Ammonifying organisms, not less than 1,000,000 and not more than 10,000,000	
Denitrifying organisms, not less than 100,000 and not more than 1,000,000	

Since the soil utilized was a good deal less than a gram (it was not practicable to weigh it because of the presence of the root and the impossibility of estimating the humidity), the positive numbers observed cannot be less than those actually existing.

This means that, after all, the flora seen under the microscope have, in all probability, no connection with living and trophic Azotobacters and Amylobacters. Since the other half of this sample had revealed under the microscope the presence of typical cluster forms, the connection of these with the Azotobacters is at least a matter of some doubt.

From such observations we have drawn the conclusion that the soil bacterial clusters may probably be considered as "cysts" functionally, analogous to those of protozoa.

This last conclusion is perhaps the most important. In so far as in a future more or less remote we might find ourselves obliged to assert that the bacteria observed by us in the soil are the only types or apparent types therein really discoverable, we now think it not illogical to suppose that in the stages of repose, a large number of the schizomycetes might tend to assume cluster-like forms, which for them would be stages of encysting, analogous to those of protozoa.

From these stages they would emerge when the soil-climate brought about modifications in the environment whereby (the cyst being, so to speak, opened) the bacteria would multiply with their characteristic rapidity, and would set up in turn that specific biochemical activity which distinguishes the species to which they belong. Although we do not claim that this is a strictly ac-

curate account, it is in any case certain that this principle of interpretation of the life history of the bacteria in the soil is borne out by the following observations:

The frequency and perhaps the universality of the cluster forms in agricultural soils;

The nearly constant form of the glomerular content, without excluding a diverse form of origin;

The relatively rare occurrence of the free bacteria in the soil;

The formation of filmy colonies wherever there is vegetable matter on the point of decomposing;

The demonstrated preservability of the soil cluster forms;

The analogy shown by the soil inhabiting cluster colonies with the colonies deep-seated in agar and gelatine cultures, an analogy which does not, however, go beyond the fact that both categories may take an almost similar form while belonging to widely differing species.

A similar interpretation leads besides to the better understanding of what is meant by a stage of equilibrium of the schizomycetic soil life, which we cannot conceive as one daily and ruthless struggle; and would tend to assimilate this life to the behavior of the protozoa, thereby unifying our concept of soil life.

Thus an explanation better than any at present available, could be given of the action, on the microbial flora, of irrigation, chemical manuring, green or stable manure, and, finally, ploughing; and a better explanation of the action of heat, cold, rain, and snow. The cluster schizomycetes would be like seeds which cultivation will cause to burst open at the proper moment.

#### CONCLUSIONS

The main conclusions that may be regarded as the result of all the work carried out so far by the authors in connection with the direct examination of agricultural soils, may be summed up as follows:

The method of staining of the microorganisms in the soil with acid aniline stains (erythrosin, eosin), first discovered by Conn and employed by Winogradsky, may be used for obtaining direct images of the whole of the microflora (and also of a part of the microfauna) found in the soil.

By the use of special methods known as *soil impression and soil crushing methods* with variants, it becomes possible to identify in the soil the existence of special forms of *bacterial colonies*, nearly always microscopic in character, which we have described as *filmy and cluster-like*. The former predominate where organic matter on the point of decomposing is present; the second are present in greater abundance in proportion as the soil is "aged" from the agricultural point of view.

The method of *burial of slides* in the soil for periods of varying length has proved valuable for the study of the soil microflora.

The number of *cluster forms* is determinable by the special method of counting.

The isolated bacteria and the bacterial clusters in the ground can be stained even 38 years after the securing of the specimen and after it is completely dried up.

Humidity and temperature may, even *in vitro*, exercise specific influences on the cluster forms.

It is probable that the soil bacterial clusters may be considered as "cysts" functionally analogous to those of the protozoa.

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# EFFECT OF DROUTH ON THE NUTRIENT LEVELS IN THE TOMATO PLANT<sup>1</sup>

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Previous tests on the tomato plant (2) showed that plants grown in a dry soil accumulated nitrate nitrogen, showed a marked lowering of phosphate phosphorus, and gained in percentage of potassium. That work was done in pots with controlled moisture. In the present work the tomato plants were grown in regular greenhouse raised benches of 8-inch depth, giving the plants a spacing of 2 feet each way. This experiment was conducted to check the previous work under more practical conditions and to obtain further data on the reliability of the plant tests as indicators of available nutrients in the soil.

## PROCEDURE

Six plants were set in each of two soil types, a red clay soil and a black silt loam. The spacing and arrangement (fig. 1) were the same for both soil types used.

Just enough water was supplied to the dry section to keep the plants from wilting during normal weather conditions. On bright days, the plants wilted, but not enough to prevent ready recovery. Enough moisture was applied to the wet section to keep the soil well moistened at all times. Excessive moisture could not collect, since a loose cypress bottom permitted ready drainage. The plants were all watered at the start until they were about a foot high, when water was reduced on the dry sections.

Samples for analysis were taken from the lower mature petioles of the plants in the wet and dry sections containing two plants each. The two plants in the medium section served merely as buffer plants. Samples from the dry plots were never taken when the plants were actually wilted. The analyses were conducted by methods previously described (1). For accurate work, a 5-gm. sample was extracted with 50 cc. of 2 per cent acetic acid instead of 10 cc. as in the field tests (1). Of this extract, 5 cc. was used for nitrate nitrogen, 10 cc. for phosphate phosphorus, and 25 cc. for potassium. A Bausch and Lomb colorimeter was used to make the color comparisons.

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

## RESULTS

*Nitrate nitrogen*

Table 1 shows that a definite concentration of nitrate nitrogen in the plants on the dry plots took place. The differences between the nitrate in the plants on dry and wet plots were greatest toward the end of the experiment. Evidently, on the dry plot, the lack of water and likely the decrease in phosphate percentage caused a lack of nitrate utilization and consequent accumulation. Apparently, the plant was able to get nitrate from the dry soil. However, nitrate absorption may have been retarded, the lack of utilization making it appear that the plant was still absorbing a large amount of nitrate.

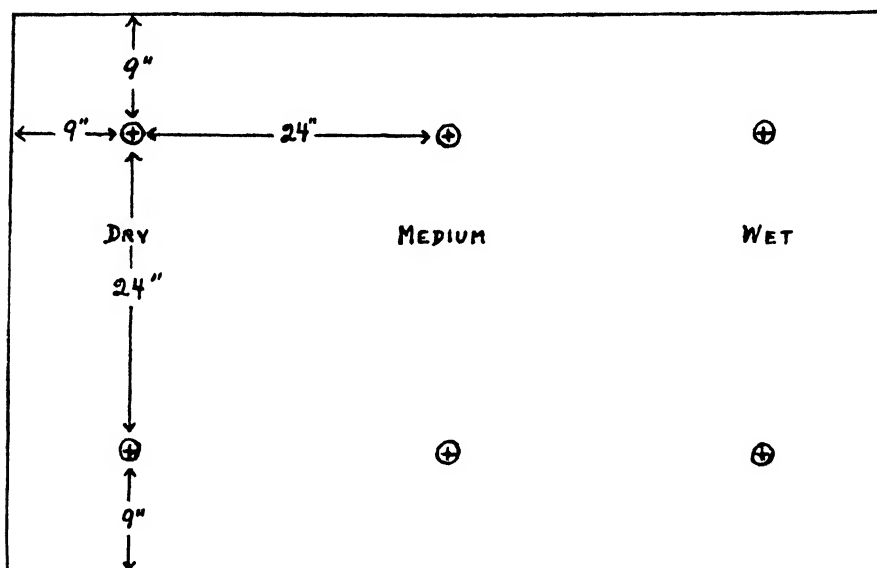


FIG. 1. SPACING AND ARRANGEMENT OF THE SIX TOMATO PLANTS IN THE GREENHOUSE BENCH

The tests also plainly show that the black silt loam was much more capable of supplying nitrate than was the red clay. The plants in the red clay soil were much smaller on both the dry and wet sections than were those on the black silt loam. The plants on the dry plots of both soil types showed the characteristic dark green color of plants containing much nitrogen and were greatly stunted. The plants on the wet plots looked normal and bore a fair crop of fruit, whereas the fruits on the plants in the dry plots were small, and practically none matured without blossom-end rot being present.

*Phosphate phosphorus*

A decrease in the concentration of phosphate in plants on the dry plots is apparent from table 2. At the time of the first and second samplings, Octo-

ber 26 and 29, plants in the dry red clay soil did not show a reduction of phosphate, whereas the black silt loam, although causing a much higher level of phosphate, showed a lower phosphate level in the plants on the dry soil at all times. Water was withheld about a week before the first sampling, but the dry plots had been dry only several days before the samples were taken. The proportion of phosphate was noticeably smaller in the plants in the dry red soil at the time of the third sampling, on November 7, and continued to decrease. On December 9, the diminution was much more marked than that caused on the dry black silt loam.

TABLE 1  
*Nitrate nitrogen in plants grown on wet and dry plots*  
(p.p.m. of fresh tissue)

TREATMENT	SOIL	OCT. 26	OCT. 29	NOV. 7	NOV. 12	NOV. 18	NOV. 28	AV. IN OCT.	AV. IN NOV.	DEC. 9
Dry.....	Black silt loam	1,408	1,146	1,210	917	555	422	1,277	776	714
Wet.....	Black silt loam	578	742	1,180	373	90	224	660	467	14
Dry.....	Red clay	122	49	176	45	35	29	86	71	23
Wet.....	Red clay	44	52	51	34	22	15	48	31	0

TABLE 2  
*Phosphate phosphorus in plants grown on wet and dry plots*  
(p.p.m. of fresh tissue)

TREATMENT	SOIL	OCT. 26	OCT. 29	NOV. 7	NOV. 12	NOV. 18	NOV. 28	AV. IN OCT.	AV. IN NOV.	DEC. 9
Dry.....	Black silt loam	273	431	275	218	114	122	352	182	214
Wet.....	Black silt loam	385	478	376	334	178	182	432	268	333
Dry.....	Red clay	69	57	43	19	25	35	63	31	19
Wet.....	Red clay	69	52	52	24	53	52	61	45	77

Some may raise the point that the plants were stunted on the dry soil and that this would cause a change in cell sap. A lack of water should concentrate the cell sap. Previous work (2), however, shows that this increase in concentration is small. It might be that a small increase in nutrients could be accounted for by the stunting effect, but in the case of phosphorus there is a decrease despite any increase in sap concentration which may have occurred. A small part of the nitrate increase may be caused by the increased concentration of cell sap but this could hardly be responsible for the large nitrate increase found. The difference in the ability of the two types of soil to supply phosphorus to the plant is brought out consistently and strikingly by the results of the plant tests in table 2.

This decrease in the phosphate level in plants in the dry soils undoubtedly was due to a lack of availability of phosphorus in the dry soil. Since the vigor-



ous plants growing in the rich black soil almost immediately showed a reduced phosphate content, the decrease could hardly be ascribed to a lack of vigor in the roots. The roots evidently continued to send up nitrate and potassium, which also would show that the roots were still vigorous in nutrient uptake, but it seems reasonable to conclude that they were unable to extract as much phosphate from the dry soil as they did from the wet soil.

### *Potassium*

Table 3 shows a consistent increase in the concentration of potassium in the plants grown in the dry soils. Just why this is the case is hard to say, but only a small part of this is likely to have been due to increased concentration of cell sap, since the water content of sap is only 1 to 2 per cent below that of plants on wet soils, as previously shown (2). The difference in the amount of potassium maintained by the two soil types is slight, the rich black soil maintaining a slightly higher amount in most cases. The fact that the concentration of potassium in plants on the black soil dropped below that in plants

TABLE 3  
*Potassium in plants grown on wet and dry plots*  
(p.p.m. of fresh tissue)

TREATMENT	SOIL	NOV. 12	NOV. 18	NOV. 28	AV. IN NOV.	DEC. 9
Dry.....	Black silt loam	3,200	3,360	4,600	3,720	3,760
Wet.....	Black silt loam	2,800	2,620	3,380	2,933	2,800
Dry.....	Red clay	2,560	3,160	4,200	3,307	3,856
Wet.....	Red clay	2,016	2,480	3,340	2,612	3,800

on the red soil in December may be ascribed to the translocation to the larger number and much larger size of fruits on the plants on the black rich soil.

### *Reliability of field tests*

Since the data reported here were obtained in a way similar to field tests for nitrate, phosphate, and potassium previously described (1), the difference being only in the amount of solvent used and the method of matching colors, it is apparent that these data give further proof of the reliability of these tests. The data are consistent in showing a reduction in phosphate and increases in nitrate and potassium on dry soils. They are also consistent in showing the differences in level of nutrients in the plant maintained by the two different types of soil used.

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# SOIL REACTION AND AZALEA GROWTH

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The azalea plant is recognized as belonging to a group of plants that require an acid soil reaction for their best growth. Thus Wilson and Rehder (8) point to the abundant occurrence of azaleas on the volcanic mountains of Japan in the absence of limestone, a condition favorable for the growth of the members of the family Ericaceae. Cox (2), in discussing the growth of the rhododendron, emphasizes that these plants prefer a soil with a certain amount of acidity, commonly provided by decaying vegetable matter. Wilson (7) warns the grower of azaleas that this plant demands a lime-free soil. Wherry (6) lists azaleas and rhododendrons as plants preferring or even requiring "subacid" soils. He designates "subacid" as a range between pH 5.1 and 6.0. Ward (5) emphasizes the fact that rhododendrons require an acid soil which is thoroughly impregnated with decomposing organic matter. Barron (1) lists azaleas as plants that will not tolerate alkaline soils. Hume (3) writes: "In common with the rhododendron, mountain laurel, andromeda, blueberry and tar flower, to which they are closely related, azaleas are acid-soil inhabiting plants. They are intolerant of lime and will not grow satisfactorily in alkaline soils or those in which lime is present in any appreciable amounts." Spencer (4) in his studies of the distribution of *Rhododendron maximum* in New Jersey did not find these plants on soils with a reaction above pH 5.6.

These reports furnish abundant evidence that the azalea plant and the closely related species require an acid soil for healthy growth. However, despite the increased use of these plants as ornamentals, neither the growth response of the plant to different degrees of soil acidity nor the type of injury attributable to alkaline soils has been studied extensively under controlled conditions. This paper reports a series of studies on the growth response of the azalea plant (*Azalea indica*) at different degrees of soil acidity and a survey of the reaction of the soils of azalea plantings of both the Indian and Kurume groups in a few of the southeastern states.

## GROWTH RESPONSE AND SOIL REACTION

Controlled cultural experiments with azaleas were started in the fall of 1931. A sandy soil with a relatively low content of organic matter and an original

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pH value of 5.76 was adjusted to different degrees of acidity by the addition of sulfuric acid or calcium carbonate. The range of soil reaction selected for study was from approximately pH 4.50 to approximately pH 8.00. Preliminary laboratory trials indicated the quantities of sulfuric acid or calcium carbonate required to obtain the desired degrees of acidity or alkalinity. The acid-treated soils were leached with tap water in the establishment of the equilibrium between the acid and the soil. By means of periodic determinations of the H-ion concentrations, the change in soil reaction was followed through the course of the experiment. Aluminium sulfate was used in an attempt to keep the acid range intact, but its use did not prevent the occurrence of an appreciable variation in the range of the H-ion concentration during the course of the experiment. The quinhydrone electrode was used to determine the H-ion concentrations in a 1-2 soil-distilled water suspension.

In October 1931, vigorous and apparently quite healthy azalea plants of the Indian group, *Formosa* variety, of uniform size and age, with the roots thoroughly cleansed of adhering soil, were planted in the soil in 4-gallon glazed pots. The pots contained 38 pounds of the soil, which had been adjusted in a series to different degrees of soil acidity. The cultures were kept in a half-shade slat shed during the course of the experiment, and were watered by the rain and by frequent applications of tap water. At intervals the plants were fertilized uniformly with liberal applications of cottonseed meal and tankage. Growth observations were made during the 2-year course of the experiment, which terminated in October 1933.

The plants were photographed at the termination of the experimental period, Plate 1, figure 1, demonstrates the outstanding response of the *Formosa* azalea plant to different degrees of soil acidity. The plants at the distinctly acid range were characterized by a slow growth and short stems between the whorls of branchlets, but the foliage was a healthy green; those at a soil pH of 6.23, by elongated growth with long stems between the whorls, and healthy green foliage; and the ones growing in a soil with an average pH of 7.58, by slow growth, distinct yellowing of the tissues between the veins of the leaves, narrow green areas of tissues along the veins, and a general unhealthy appearance indicative of a lack of thrift.

A bed of azaleas of the *Formosa* variety growing on the horticultural grounds of the Florida Agricultural Experiment Station was used as a correlation in definitely determining the limits of alkalinity which bring about the chlorosis of azalea plants. The soil was a Norfolk medium fine sand very similar to that used in the pot experiments. Accidentally, through the dumping of spray materials, a range of reaction had been brought about in the soil in different parts of the bed, and the plants had grown under these conditions for several years.

Three distinct plant conditions, as shown in plate 1, figure 2, were found to exist in this bed. The sprig on the left was from a plant growing in a soil with a reaction of pH 6.52, the middle sprig with partially chlorotic foliage

was from one growing in a soil with a reaction of pH 7.07, and the sprig on the right was from one in a soil having a pH of 7.31. The distinctly chlorotic condition of the plant growing in a soil having a reaction of pH 7.31 should be emphasized. The green veins and the yellowed areas between are characteristic of azalea plants growing in a distinctly alkaline soil, containing carbonates. These results suggest that the danger zone, as far as chlorosis in azaleas caused by an alkaline soil is concerned, lies within the narrow limits of soil acidity represented by a pH range between 6.50 and 7.00.

These field studies, combined with the pot cultures as described, give a basis for a more definite determination of the growth response of azaleas to the different degrees of soil acidity.

Plate 1, figure 3, shows the entire series of pot cultures and indicates the growth response in the Formosa variety that may be expected at different degrees of soil acidity. Growth was slow, and both the stem length and the internodes between the whorls of branches were definitely shortened at a soil pH value lower than 5.00. However, the foliage was dark green and the plants appeared healthy and flowered normally. With the Formosa variety, and perhaps others, it appears that the soil acidity should be kept below pH 5.00 where low-growing, bunchy plants are desired. An intermediate type of growth was observed between pH 5.00 and 6.00. Here, the stems between the whorls of twigs were elongated, and twig growth was lengthened. This condition produced a larger but yet not spreading plant, which without doubt is suitable for many uses in the garden. The foliage was healthy and green and the flowering normal in this range. Within a soil reaction range represented by a pH value of about 6.00 to slightly above a pH value of 6.50, the growth was rapid with distinctly elongated stems between the whorls of branches. This condition resulted in an open, somewhat sprawling type of plant. Foliage was healthy and flowering normal. Above a soil reaction of pH 7.00, shortened growth with distinctly chlorotic leaves, sparse foliage, and a general unhealthy plant condition were observed. In this range the plants flowered sparsely. The range of reaction represented by pH values between 6.50 and 7.00 may be considered a danger zone in which azalea plants, although not without exception, are likely to become chlorotic.

The response of these azalea plants to different degrees of soil acidity under controlled conditions made a survey of the soil reaction of azalea plantings of particular interest. Such a survey was undertaken during the winter and spring of 1933-34.

#### SURVEY OF THE REACTION OF THE SOILS OF AZALEA PLANTINGS

As it was not feasible to visit and sample the soils of the numerous azalea plantings, requests were made of a number of friends and growers of azaleas to collect soil samples and forward them to Gainesville, where the H-ion determinations were made. Table 1 gives the names of the areas sampled, the number of locations sampled, and the number of samples taken. In all, 21

areas, 63 locations, and 168 azalea soils were sampled. Samples from the famous planting in the Magnolia Gardens at Charleston, S. C., as well as from the oldest plantings in Florida were included. Several soil samples also were taken from wild species.

As a rule the samples were taken to a depth of 12 inches—a soil depth which probably contains the greater portion of the root systems of the plants. The samples were collected underneath the growing plants. Since the soil samples were collected from different depths by the various collectors, the H-ion concentrations of the different depths have been averaged for any one soil. All

TABLE 1  
*List of areas where soil samples from azalea plantings were collected*

AREA	LOCATIONS	NUMBER OF SAMPLES
Charleston, S. C.....	2	19
Savannah, Ga.....	2	3
Macon, Ga.....	1	1
Mobile, Ala.....	4	9
Marianna, Fla.....	1	2
Quincy, Fla.....	4	4
Tallahassee, Fla.....	2	6
Monticello, Fla.....	5	18
Lake City, Fla.....	1	3
Glen Saint Mary, Fla.....	5	10
Jacksonville, Fla.....	7	15
Hastings, Fla.....	1	3
St. Augustine, Fla.....	2	2
Deland, Fla.....	3	9
Gainesville, Fla.....	5	6
Leesburg, Fla.....	3	8
Tampa, Fla.....	1	6
Bradenton, Fla.....	1	1
Winter Park, Fla.....	3	6
Winter Haven, Fla.....	3	25
Lake Wales, Fla.....	7	12
Totals..... 21	63	168

H-ion concentrations were determined in the same manner as used for the pot experiments. It was impossible to designate the different soil types, as in a majority of the cases the soils had been especially prepared for azalea culture by additions of mucks or other soils and vegetable litter.

The results of the determinations of the pH values of the azalea soils are shown graphically in figure 1. The graph illustrates the distribution of these soils with regard to soil reaction. As may be noted, the greatest number (38.7 per cent) of the azalea soils were found to have a pH value between 5.00 and 6.00, a range of H-ion concentration evidently very favorable for

the growth of azalea plants. In 67.8 per cent of the soils the reaction was lower than pH 6.00. In 26.8 per cent of the samples the reaction was between pH 6.00 and 7.00. Of these last-named samples, 16.7 per cent were found to be between pH 6.00 and 6.49, and 10.1 per cent were found in the range of pH 6.50 to 6.99, a range previously designated as the danger zone for chlorosis. In all, 15.5 per cent of the soils were in the zone of possible injury from chlorosis, and 29.1 per cent of the plants were growing in very acid soils (lower than pH 5.00). The most acid soil had a pH value of 3.54; the most alkaline,

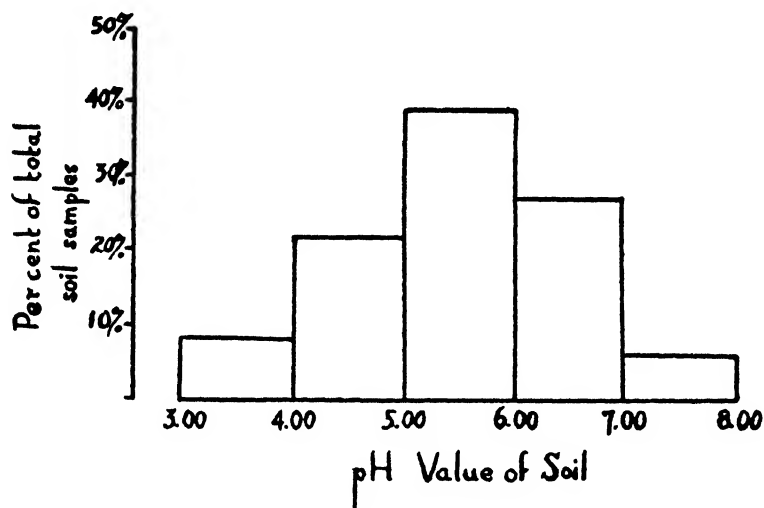


FIG. 1. DISTRIBUTION OF SAMPLED AZALEA SOILS WITH REGARD TO pH VALUES

TABLE 2

*Reported condition of azalea plants at different degrees of soil acidity*

PLANT CONDITION	pH RANGE OF SOILS					
	3.00-4.00	4.00-5.00	5.00-6.00	6.00-6.50	6.50-7.00	7.00-8.00
Number of soils sampled.....	12	37	65	28	17	9
Percentage of plants reported healthy.....	75	76	75	71	47	22
Percentage of plants reported unhealthy.....	25	24	25	29	53	78

a value of 7.48. The acid soil was collected from a drained cypress swamp; the alkaline soil, near a building where lime materials had accumulated. Observations on the growth condition of the plants were made at the time the soil samples were collected.

The notes on plant thrift are summarized in table 2, where the percentages of healthy and of unhealthy plants are given for each reaction range of the soil. From the table it may be seen that up to a soil pH value of 6.50, approximately 75 per cent of the azalea plants were reported in a healthy condition.

The range of soil pH values between 6.50 and 7.00 is marked by an abrupt change of plant condition, wherein only 47 per cent of the plants were reported to be healthy. Above a soil pH value of 7.00 only 22 per cent of the plants were reported as being in a thrifty condition—in other words, two only of the nine sampled. In this last range, the two samples of soils reported from healthy azalea plants were from specimens growing in a soil with a pH value of 7.20—slightly above the neutral point. Where a soil reaction above pH 7.00 was accompanied by an unhealthy plant condition, the plants in every instance were reported as being chlorotic. Many of the plants growing on soils with a reaction between pH 6.50 and pH 7.00 were reported as chlorotic. The rapidity with which transplanted azalea plants are affected by soil reaction apparently is in direct ratio with the degree of acidity or alkalinity of the soils in question. Thus on a soil with a nearly neutral reaction, plant vigor would be more slowly checked than on a strongly alkaline one, highly alkaline soils reacting adversely within a comparatively short time. The results of this survey corroborate those obtained in the control experiments.

#### SOME ADDITIONAL FACTORS AFFECTING AZALEA GROWTH

Factors other than soil reaction were reported as bringing about an unhealthy growth of the azalea plant. A few of these factors and their effects on the growth of the azalea plant will be briefly discussed.

*Poor Soil Drainage.*—Although the azalea is recognized as a plant that requires a great deal of water for its growth, it cannot be considered as one that will grow in a poorly drained soil. Several instances of plants suffering from poor drainage were reported in the survey of the soils of azalea plantings. In one instance where the water table was only 10 inches below the surface, the plant had an appearance not unlike those affected with lime chlorosis, although the soil was sufficiently acid in reaction. Small leaves with yellow areas between the veins were observed, and the plant was definitely stunted in growth. In other instances of excessively poor drainage, plants were reported as being virtually killed by the suffocation of the roots in the saturated soils.

*Drought.*—A distinct curtailment of the water supply first causes extreme wilting of the foliage and soon brings about a dying back of twigs and a definite lack of thrift in the plant. In common with numerous plants growing in humid regions, the azalea seemingly to a degree adjusts itself to a limited supply of water by a corresponding limitation of root and top growth but suffers acutely from extended periods of little moisture. Several instances of drought injury were reported in the survey.

*Fertilization.*—An unhealthy plant condition brought about very likely by inadequate fertilization was observed in the survey. A very light green foliage, which is commonly characteristic of an insufficiency of nitrogen in many plants, was noted in several instances. The azalea plant usually is greatly benefited by liberal applications of commercial fertilizing materials, which in most cases

should be from sources that will tend toward the creation of a more acid soil condition.

*Cold Damage.*—Symptoms of cold injury are in nowise similar to those due to an unsuitable soil reaction and there is little likelihood of confusing the two. Frost injury may not be apparent until after a lapse of several weeks from the time of the actual freezing and may be characterized by rather sudden wilting of the foliage on a twig, branch, or even the whole plant, followed by a browning of the leaves and later death of the affected parts.

#### SUMMARY

1. Formosa azalea plants were grown in a soil adjusted to different degrees of soil acidity. Below a degree of acidity represented by pH 5.00, the plants made a slow but healthy growth under the conditions of these experiments. Between pH 5.00 and 7.00, the plants made a vigorous, healthy, elongated growth. The plants grown between pH 5.00 and 6.00 were especially healthy with desirable growth characteristics. Above pH 7.00, the growth was slow and the foliage chlorotic.

The chlorosis of the azalea which is produced by an alkaline soil may be described as a yellowing of the tissue of the leaves between the veins. The veins remain green. Chlorotic leaves are usually smaller than healthy green leaves.

2. A survey of azalea soils included 168 samples of soil taken from the first foot of azalea plantings in several southeastern states. Of the soils, 94.6 per cent had pH values lower than 7.00. The range of pH values between 5.00 and 6.00 had the largest number of the samples (38.7 per cent). Approximately 75 per cent of the plants were reported healthy when the soils had a pH value lower than 6.50; 47 per cent of the plants were reported healthy in a pH range between 6.50 and 7.00; and only 22 per cent were reported healthy when the pH value of the soils exceeded 7.00. The chlorosis induced by an alkaline soil was responsible for the lack of thrift in the majority of the unhealthy plants reported for soils with pH values above 7.00.

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## PLATE 1

## EFFECT OF SOIL REACTION ON GROWTH AND FOLIAGE OF AZALEA

FIG. 1. The outstanding growth responses of the Formosa azalea plant to different degrees of soil acidity. Uniform plants set October 1931; photographed October 1933.

Left—average pH 4.78; slow growth; stems between whorls shortened; foliage green; flowering normal.

Middle—average pH 6.23; more rapid growth; stems between whorls elongated; foliage green; flowering normal.

Right—average pH 7.58; slow growth; practically all leaves chlorotic; flowering sparse.

FIG. 2. Limits of alkalinity bringing about a chlorotic condition of azalea foliage on a sandy soil.

Left—green foliage. Five soil samples, average at depths of 0-2 inches, pH 6.39; 2-6 inches, pH 6.63; 6-12 inches, pH 6.64; average all depths, pH 6.52.

Middle—partially chlorotic foliage. Four soil samples, average at depths of 0-2 inches, pH 7.04; 2-6 inches, pH 7.07; 6-12 inches, pH 7.11; average all depths, pH 7.07.

Right—chlorotic foliage. Three soil samples, average at depths of 0-2 inches, pH 7.34; 2-6 inches, pH 7.36; 6-12 inches, pH 7.29; average all depths, pH 7.31; carbonates present in all samples.

FIG. 3. Growth response of the Formosa azalea to different degrees of soil acidity. Plants of uniform size and age set October 1931; photographed January 1934.

	1	3	4	5	6	7	8	9	10	11	12	13	14
Average pH.....	4.78	4.82		5.65		6.23		6.49		6.61		7.58	
Average spread...	44 in.	41 in.		52 in.		58 in.		52 in.		57 in.		35 in.	
Average height...	25 in.	34 in.		38 in.		37 in.		42 in.		31 in.		28 in.	
	Range of slow growth. Bunchy plants. Healthy green foliage. Natural flowering			Range of elongated growth—usually more elongated under less acid conditions. Healthy green foliage. Natural flowering				Range of danger of lime chlorosis			Range of slow growth and chlorotic foliage. Sparse flowering		
pH.....	3.00			5.00						6.50		7.00	8.50

R. M. BARNETTE AND HAROLD MOWRY



FIG. 1



FIG. 2



FIG. 3



# THE ORIGIN AND SIGNIFICANCE OF AMMONIA FORMED BY AZOTOBACTER

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## INTRODUCTION

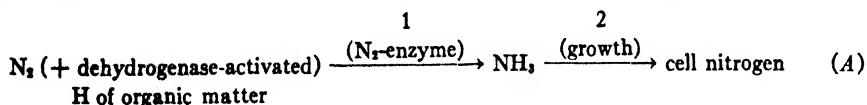
### *Historical*

In the voluminous literature which accumulated on *Azotobacter* during the first quarter of a century after its discovery in 1901, there are to be found only a few sporadic reports which record the occurrence of small amounts of ammonia in pure cultures of the organism. The conditions governing this occasional, almost doubtful, formation of ammonia were never investigated, and a general interpretation of the phenomenon remained in complete obscurity.

Some 10 years ago, however, Kostytshev and his co-workers (18, 19, 20, 22, 23) reported extensive solution culture experiments with *A. "agile"* (17) in which easily measurable quantities of ammonia and amino acids were consistently obtained in the culture medium separated from the cells and pellicles. This work, standing in marked contrast to an enormous number of previous investigations, immediately challenged attention, the more so because apparently no strikingly new cultural methods had been employed. For some time it was believed that the positive results of Kostytshev were explicable mainly upon a basis of strain specificity or culture impurity. In fact, a number of workers of the Russian school, including Demidenko (10), Minenkov (27), and Novogradsky (28), using *A. chroococcum*, were unable to repeat Kostytshev's experiments. This was true even though additional, very delicate and somewhat novel methods for detecting ammonia were employed.

Eventually Winogradsky (32, 33, 34), using silica gel media, succeeded in obtaining cultures of various strains of *Azotobacter* which readily produced ammonia when allowed to become alkaline as a result of growing upon alkali salts of organic acids, the latter being preferentially absorbed and leaving a residue of strong alkali. The greatest yields appeared to be obtained in the region of pH 9.0. Comparatively neutral organic salts, such as those of calcium, yielded no ammonia. Small amounts of ammonia were obtained under neutral conditions with cultures grown upon alcohol, but essentially none with mannite or sugar. Other specific, less effective conditions found to favor ammonia liberation by cultures were depletion of substrate; exposure of cultures to vapors of antiseptics such as ether, chloroform, or toluene; emulsification of cells in glycerine; and mechanical destruction of the organisms.

In interpreting his results, Winogradsky concluded that the ammonia liberated by the cells into the culture medium—and, under appropriate conditions, partially distilled spontaneously therefrom—was produced chiefly as a result of a disturbed, delicate balance between two processes: 1, a primary synthesis of  $N_2$  to  $NH_3$  and 2, an assimilation of this  $NH_3$  in growth and metabolism. His view may be represented schematically in detail as follows:

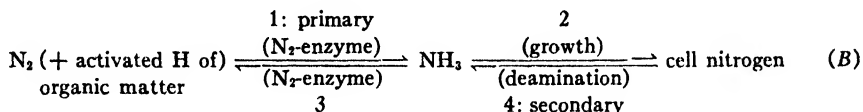


Winogradsky held that steps 1 and 2, although normally in perfect equilibration, were in reality independent, and could be separated under various suitable conditions, the most no-

table and demonstrable being high alkalinity. Any relative retardation of the life processes represented by step 2 would result in the liberation of (unutilizable)  $\text{NH}_3$  by way of step 1. It was even suggested, upon the basis of the experiments with antiseptics and cell maceration, that step 1 might proceed in the entire absence of life processes. Autolytic or deaminative formation of ammonia was considered to be rare and negligible, except possibly in extremely old cultures.

Kostytshev had earlier presented much the same scheme or view (19), except for considering, as an additional feature, that step 1 was freely reversible, in accordance with the mass law. He reached this conclusion upon finding that addition of  $\text{NH}_3$  to cultures prevented utilization of  $\text{N}_2$ . He believed that nitrate acted in the same way, although somewhat less effectively per unit of nitrogen, by virtue of being reduced to  $\text{NH}_3$ ; and that peptone also acted likewise, but still less effectively, by virtue of small quantities of  $\text{NH}_3$  contained as impurity. He concluded from his experiments, as did Winogradsky later, that  $\text{NH}_3$  is the "first" product of  $\text{N}_2$ -assimilation by *Azotobacter*.

In his last published paper, based on studies with *A. vinelandii*, Kostytshev (21) arrived at the further modified conclusion that step 2 was to some extent reversible also. His final scheme, worked out with Scheloumova, may be represented as follows:



Kostytshev and Scheloumova thus held that there were really two important methods of ammonia formation: (primary) direct synthesis from  $\text{N}_2$  which occurred only when organic substrate (sugar) was present, and (secondary) deamination of cell nitrogen which occurred only when organic substrate was absent. Under all conditions observed, the secondary formation of ammonia was, in point of fact, greater than the primary, usually by a factor of several fold. In support of the secondary formation, he emphasized especially that no  $\text{N}_2$ -assimilation took place after the disappearance of the organic substrate; and in support of the primary formation he found that deamination of peptone and glycine took place in the absence, but not in the presence, of organic substrate, from which he concluded that cell nitrogen also would not yield ammonia in the presence of sugar. In this connection, however, Uspensky (30) has since suggested that deamination of non-living material is one thing, whereas splitting off of ammonia from live protein is still another.

Isakova (15) recently reported experiments with both *A. vinelandii* and *A. chroococcum* which confirm those of Kostytshev and Scheloumova (21) in showing the production of ammonia in neutral or only very slightly alkaline media containing mannite or glucose, as well as salts of organic acids. In the light of these results, Winogradsky's view as to the relative importance of extreme alkalinity in ammonia formation appeared to require considerable modification.

The recent note of Bach, Yermolieva, and Stepanian (2), reporting the production of  $\text{NH}_3$  from  $\text{N}_2$  and glucose by "non-living," cell-free press juice extracts of *Azotobacter* cells, and awaiting extension and confirmation, is for the present beyond the immediate considerations of this paper, which deals with ammonia formed by or from *Azotobacter* cells; eventually a close relationship between the two investigations might develop.

### *Critique of previous investigations*

The investigations just described evidently possess an important agronomic significance, in demonstrating the potentially immediate, fertilizing rôle of *Azotobacter*, long held highly questionable because of the very slow decomposition of these organisms by well-known microbiological agents.

A second, equally important aspect of the observed evolution of ammonia by cells of *Azotobacter* is its possible relation to the chemical mechanism of nitrogen fixation by these organisms: Is, or is not, ammonia a major, essential intermediate product formed in the complicated process whereby gaseous  $N_2$  is converted into organized cell nitrogen? Unfortunately, for a number of reasons, the experimental findings cited, although at first sight suggestive in this connection, cannot be said to provide any conclusive answer to this difficult question. It will be very desirable at this point to indicate some of the more immediately evident criticisms; others will be postponed until discussion of the experimental results presented in this paper. The chief inherent weaknesses of the earlier investigations may be said, in general, to lie in their essentially qualitative, rather than quantitative outlook, and in their failure to include certain clearly necessary control experiments, and certain very desirable types of related experiments to be mentioned shortly.

In the previous investigations the cultures were at no time placed in an atmosphere devoid of nitrogen gas, in order to determine the necessity for the presence of  $N_2$  for  $NH_3$  formation, that is to say, for the actual simultaneity of  $NH_3$ -formation with  $N_2$ -disappearance. Nor were studies with various qualitative and quantitative mixtures of oxygen, nitrogen, and inert gases (i.e.,  $H_2$ ) carried out to determine the various influences of such gases.

It was not shown that cultures grown in a considerable variety of fixed nitrogen compounds did not likewise give off  $NH_3$ , and thus establish a reasonable specificity for the evolution of  $NH_3$  from cultures grown with free  $N_2$ . Kostytshev performed a few such experiments with nitrate, which will be considered later.

The capacity of mature cultures, grown in either free or fixed nitrogen, to ammonify a wide variety of added, known fixed nitrogen compounds, particularly those types of probable occurrence in *Azotobacter* cells, was not determined to any extent.

The course of sugar consumption was not followed quantitatively in relation to ammonia formation, nor was respiration—oxygen consumption or carbon dioxide production—studied. The relative importance of these probably very important factors was thus impossible to assess.

Finally, the technique employed was in general inadequate to determine reasonably closely the optimum conditions for ammonia production, with respect to important variables such as pH, temperature, anaerobiosis, added organic matter, cell concentration, and inhibiting agents. The chief reasons for this lay in the heterogeneity of the solid, or pellicle-liquid, stagnant cultures employed, involving diffusion limitation, and non-uniformity and uncertainty of concentration of various nutrients, from one cell to another in a given culture. It was therefore relatively difficult either to impose or to measure, with desirable accuracy, the environmental conditions, which varied in different regions of one and the same culture. The subtle bearing of this circumstance should be fully appreciated when interpreting findings heretofore obtained.

### *Statement of problem*

In this and two accompanying papers (7, 14) are reported, with due appreciation of the criticisms just advanced, the results of a detailed and widely varied investigation directed in the interest both of obtaining many further details of agronomic import concerning the formation of ammonia by *Azotobacter* and with a view to ascertaining, so far as possible, whether the ammonia observed is liberated *before* a synthesis into normal cell nitrogen, as in schemes A

or B of Winogradsky and Kostytshev, or *after* a synthesis into normal cell nitrogen. Is none, some, or all of the extracellular  $\text{NH}_3$  *which has been observed* derived from cell nitrogen, or to what extent is it derived specifically, uniquely, and directly from  $\text{N}_2$ ?

## EXPERIMENTAL

### *General methods*

**Cultures and media.** The methods of culture, and the Warburg technique used to measure oxygen consumption, carbon dioxide production, and respiratory quotient, have been previously described in considerable detail (3, 5, 9, 13, 25). The "stock cultures," used for purposes of inoculation, or in the preparation of "stock suspensions," have been grown in an air thermostat at  $28^\circ\text{C}$ . in either 250-cc. gas wash bottles containing 100 cc. of (sterile) culture medium, or 2.5-liter reagent bottles containing 2 liters of medium, aerated with air (or occasionally 50 per cent  $\text{O}_2$  in  $\text{N}_2$ ) at the rate of 25 to 200 cc. per minute. The culture medium consisted of 1 to 2 per cent glucose (or sucrose) and 0.00025 gm.  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  and 0.005 gm. synthetic (sugar) humate containing 0.0005 gm. Fe, made up in the clear, liquid "inorganic medium" obtained after the following mixture had been thoroughly shaken, allowed to stand, and settle: 0.8 gm.  $\text{K}_2\text{HPO}_4$ , 0.2 gm.  $\text{KH}_2\text{PO}_4$  (pH 7.2), 0.2 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 gm.  $\text{NaCl}$ , 0.1 gm.  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , and 1000 gm.  $\text{H}_2\text{O}$ . The clear solution contains about 15 per cent less phosphate and 40 per cent less calcium than the unsettled liquid. Unless otherwise indicated, this "inorganic medium" was employed in making up all cultures, sometimes being fortified, for greater buffer action, with addition of 3 to 10 times the normal phosphate mixture of the desired pH.

The "stock suspensions" were prepared by centrifuging the "stock cultures" under refrigeration in 500-cc. bottles at 2000 r.p.m., or occasionally in the Sharples supercentrifuge. The material was then washed and centrifuged twice with inorganic medium diluted fivefold with distilled water, and finally taken up in this same diluted medium at a concentration 10 to 100 times the original cell concentration, generally 5 to 25 billion per cubic centimeter (about 4 to 20 mgm. cell dry matter per cubic centimeter, or 1 to 5 mgm. cell N per cubic centimeter (table 10)). These suspensions were stored at  $5^\circ\text{C}$ . until needed and would remain essentially unchanged for over a month, with negligible  $\text{NH}_3$ -formation and no decrease in respiration capacity (tables 10 and 11). For use in the Warburg vessels the stock suspensions of organisms were diluted two- to tenfold with inorganic medium diluted fivefold with distilled water, but fortified with 3 to 10 gm./l. of phosphate buffer. Apart from concentrating the material and renewing the inorganic medium, no effect, physical, chemical, or physiological, ascribable to centrifuging carried out as indicated, has ever been observed concerning the behavior of the cells (table 9). No significant growth or contamination was ever observed to develop in these suspensions while maintained at low temperature and containing neither fixed nitrogen nor organic substrate.

As gas phases, there were employed, in addition to air, pure oxygen and various (nitrogen-free) mixtures of  $\text{O}_2$  in  $\text{H}_2$ , prepared by means of a multiple flowmeter (3); and also, to provide for anaerobic conditions, pure  $\text{H}_2$  or pure  $\text{N}_2$  passed through alkaline pyrogallol to eliminate traces of  $\text{O}_2$ . These various gases and mixtures were employed with both cultures and suspensions in both Erlenmeyer and Warburg microrespiration flasks.

The respiration rates in the Warburg apparatus were usually measured hourly for the first 1 to 6 hours, then possibly overnight, if the magnitude permitted, otherwise the manometers were left open to the air. The same procedure was followed on second, third, and later days, when involved. The  $\text{QO}_2$ , or cubic millimeters  $\text{O}_2$  consumed per milligram of cell dry matter per hour, was usually calculated from readings several times larger than its own magnitude (several milligrams dry matter being involved). Various values reported, particularly in tables 10 and 11, permit an estimate of the usual accuracy attained.

Three strains of *Azotobacter* were studied: *A. vinelandii*, a vigorous nitrogen-fixing organism, obtained originally from the New Jersey Agricultural Experiment Station and used in this laboratory for many years; *A. chroococcum*, a stock culture obtained through the courtesy of N. R. Smith of the Bureau of Plant Industry; and *A. chroococcum* Strain S.M. 1, obtained in 1927 from the Rothamsted Experimental Station and isolated from a Harpenden soil.

**Analytical methods.** The pH was determined either colorimetrically, with one or more La Motte standards, or electrometrically, with a Welch quinhydrone apparatus.

Glucose was determined in some cases by the well-known Bertrand, copper reduction method, which is not affected by the presence of *Azotobacter* cells. Ordinarily, however, glucose was determined by a method worked out using Nessler's reagent. At concentrations greater than 0.001 to 0.005 per cent glucose, this reagent gives a red precipitate, which is photolabile and will turn yellow or black as the surrounding light intensity is greatly increased. The precipitate is given also by fructose, maltose, lactose, l-arabinose, and l-xylose, but not to any extent by sucrose, mannite, succinate, lactate, benzoate, acetate, or glycerophosphate. There is normally an induction period of at least 5 to 20 seconds, depending upon the concentration, before any precipitate develops at all, and 15 to 45 minutes are required for complete precipitation. *Azotobacter* cells have never been observed to give any reaction themselves but, when present in high concentration, may appear to hasten the reaction given by glucose, and in this case it is preferable to have the standards contain (glucose- and  $\text{NH}_3$ -free) cells also. In making the test 0.1 to 0.3 cc. of unknown are added to 1 cc. of Nessler's reagent prepared by the method of Army and Ring (1), and compared against standards. This convenient method of determining the order of concentration of glucose (etc.) has not, to our knowledge, been reported hitherto.

$\text{NH}_3$ -N was determined by microdistillation or by Nesslerization, with concordant results if both methods were used (tables 7 and 16). The Nesslerization was carried out with 0.1 to 0.3 cc. unknown added to 1 to 2 cc. reagent, with cells in the standards where very high concentrations produced shading. The unknowns were always diluted to contain 10 mgm./l.  $\text{NH}_3$ -N or less, so as to obtain color changes only, and no precipitation. The reaction product was not photolabile, and was formed in a few seconds, so that normally the subsequent formation of precipitate by any glucose present did not interfere; nor, conversely, did  $\text{NH}_3$ -N affect the glucose test, which was normally one or more orders more intense. The  $\text{NH}_3$ -N determinations were usually accurate to  $\pm 5$  to 10 per cent, with  $\pm 25$  per cent as an occasional maximum. The relative order of accuracy was arbitrarily varied in accordance with the relative importance of a given experiment. Needless to say, where necessary, the determinations were corrected for blank and control values. The  $\text{NH}_3$ -N concentrations in undiluted cultures ranged, in absolute magnitude, up to 500 mgm./l., so that with determinations at 1 to 5 mgm./l an accuracy of about 1 per cent was attained for the order involved. Microdistillation provided a somewhat greater relative accuracy than Nesslerization, and was carried out for 5 to 10 minutes in a modified Pregl apparatus. The cells themselves did not decompose to provide  $\text{NH}_3$ -N under these conditions. In several hundred distillations performed during the past 7 years on cultures which had been grown in either  $\text{N}_2$ -N, nitrate-N, or  $\text{NH}_3$ -N, for 1 to 180 days, no traces of  $\text{NH}_3$ -N (0.2 mgm./l. or less) were ever obtained, even after distillations of from 10 minutes to 2 hours, provided there was no  $\text{NH}_3$ -N in the medium surrounding the cells, as indicated by negative Nessler's test carried out at ordinary temperatures.

Growth was measured in four ways. Total nitrogen was determined with the macro-Kjeldahl method, carried out by Mrs. E. Rist, or with the micro-Kjeldahl method, carried out by Dr. R. T. Milner and Mrs. M. S. Sherman, of the Analytical Division of this Laboratory. Cell-dry matter was determined by centrifuging aliquot portions of culture in weighed cups, discarding the clear liquid, drying at  $100^\circ\text{C}$ ., and weighing. Turbidimetric measurements with arbitrary standards were made visually or turbidimetrically with a Bausch and Lomb nephelometer. Cell counts were made with specially constructed bacterial haemocytometers of 0.002 mm. depth.



### *Preliminary studies*

*Confirmation of previous work.* Our first efforts were directed toward repeating the previously published work. As a variant in procedure, it was found very simple and convenient to grow cultures in shallow layers of liquid in Erlenmeyer or Roux flasks, with occasional shaking by hand to prevent pellicle formation, and to maintain a reasonably uniform distribution of cells. A general confirmation of the earlier, positive experimental findings was easily obtained with this technique. Cultures growing in common inorganic media containing *initially* 0.1 to 3 per cent organic substrate readily yielded ammonia after varying periods of incubation under appropriate conditions. The organic substrates studied were glucose, sucrose, and the sodium salts: succinate, benzoate, acetate, butyrate, lactate, and malonate. In several instances the yields of  $\text{NH}_3\text{-N}$  obtained were 30 to 40 per cent of the total cell nitrogen involved and were thus somewhat greater than any reported previously. The actual concentrations of  $\text{NH}_3\text{-N}$  found in the medium usually ranged up to 10 mgm./l. (10 p.p.m.), but in some cases attained 30 to 40 mgm./l., even in essentially open (cotton-stoppered) flasks, where loss by evaporation often amounted to 10 to 50 per cent.

The best results were obtained with initial mixtures of 0.5 to 2 per cent glucose and 0.2 to 0.5 per cent organic alkali salt. The sugar provided a greater increase in cell mass (growth), and the organic salt a more favorable, alkaline reaction. With sufficient organic alkali salt, the pH rose rapidly to 9 and, with an excess, attained at times a value of 11, even in the presence of the carbon dioxide of the air. Little if any growth or nitrogen fixation was observed to take place above pH 9.0 to 9.3, or after the formation of  $\text{NH}_3$  had commenced, or, in accordance with the findings of Kostytshev and Scheloumova (21), after the disappearance of the organic substrate.

Tables 1 to 8 are illustrative of the methods and results obtained in 73 out of a total of over 300 cultures involved in the preliminary study. Because of their exemplary or confirmatory nature, and because the later, more refined work will bring out all the new major points more clearly, these tables will not be analyzed individually except for the notes appended to them. However, the following general variables of procedure or situation may be noted in particular: sources and initial concentrations of organic substrates (tables 1, 2, 4); residual concentration of glucose (tables 3, 4, 5); nitrogen substrates for growth (tables 2, 3); pH (table 7); residual (essentially cell-free) medium (table 6); marked evaporation of  $\text{NH}_3\text{-N}$  (tables 2, 6, 7, 8) and method of stoppering (table 1); relative and absolute volumes of culture media and flasks (tables 2, 4); size and kind of inoculum, including species; duration of incubation and onset of  $\text{NH}_3$ -formation, course of  $\text{NH}_3$ -formation and pH change; temperature.

*New findings.* In addition to confirmatory features, several new findings obtained in the preliminary study, besides the increased yields of  $\text{NH}_3\text{-N}$  already noted, should be observed. The replacement of 78 per cent  $\text{N}_2$  in  $\text{O}_2$

by 78 per cent  $H_2$  in  $O_2$ , without alteration in  $NH_3$ -formation, qualitatively or quantitatively (table 1), is highly indicative of the non-existence of an immediate relationship between  $NH_3$ -formation and  $N_2$ -disappearance, and has a marked negative bearing in connection with schemes A and B. The equivalent replacement of  $N_2$  by nitrate-N, as a source of nitrogen for growth and  $NH_3$ -

TABLE 1

*Influence of glucose, concentration of succinate, and hydrogen-oxygen gas on the course and extent of  $NH_3$ -formation*

INITIAL SUBSTRATE		DAYS						
		0	1	2	3	4	5	
		Gas phase						
		Air	Air	Air	Air	Air	21 per cent O <sub>2</sub> in H <sub>2</sub> from 3rd day*	Air
A. 1 per cent glucose	pH NH <sub>3</sub> -N	7.0 0	7.0 0	7.0 0	7.0 0	7.0 0	7.0 0	7.0 0
B. 2 per cent Na-suc- cinate	pH NH <sub>3</sub> -N	7.0 0	7.3 0	7.8 0	9.0 0	9.3 0	... ...	... 0
C. 0.7 per cent Na-suc- cinate	pH NH <sub>3</sub> -N	7.0 0	7.4 0	8.2 0	8.8 1.4	9.3 26	9.3 27	... 40
	Total-N NH <sub>3</sub> -N Total-N', per cent					127 21	128 21	(127) 32

25 cc. inorganic culture medium in 750-cc. closed (rubber-stoppered) Erlenmeyer flasks, inoculated with 5 cc. of 6-day-old, aerated stock *A. vinelandii* culture in which original glucose was nearly consumed. Temperature, 34°C.  $NH_3$ -N (microdistillation) and total-N (Kjeldahl) in mgm./l.; pH colorimetric.

\* A and C cultures each divided on third day (when  $NH_3$ -formation was just commencing in C culture) into two aliquots then placed in air and in 21 per cent  $O_2$  in  $H_2$ , respectively.

Notes: The absence of  $NH_3$  in the 2 per cent succinate culture as compared with the 0.7 per cent succinate culture, *in spite of similar courses of pH change*, is ascribable to substrate presence in the former even after 5 days, but its disappearance by 3 days in the latter. Presence of substrate, and to some extent low pH, prevented  $NH_3$ -formation in the 1 per cent glucose culture. Growth was heavy in all three cultures, but somewhat differentiated in the decreasing order, 1 per cent glucose, 0.7 per cent succinate, 2 per cent succinate. The replacement of  $N_2$  by  $H_2$  in the (21 per cent  $O_2$ ) gas phase has effected no change in the extent or course of  $NH_3$ -formation in either the glucose or 0.7 per cent succinate cultures.

formation (table 2), has a similar significance. Both these findings will be still further enlarged and generalized, but it is to be noted here that in the particular experiments on which these findings are based  $NH_3$ -formation was actually rather large; consequently their significance and relation to previously published work is in any case assured.

TABLE 2

*Influence of source of nitrogen for growth, and succinate concentration, on NH<sub>3</sub>-formation*

N-SOURCE FOR GROWTH	CUL-TURE	FLASK	SUC-CINATE		DAYS						
	cc.	cc.	per cent Na-salt		0	2	3	4	6	7	12
N <sub>2</sub> -N	6	150	1.5	pH	7.0	9.31	9.5	9.73	9.8	10.0	....
				NH <sub>3</sub> -N	0	0	0.2	0.4	0.4	....	....
			1.0	pH	7.0	9.14	9.4	....	9.8	....	10.0
				NH <sub>3</sub> -N	0	1	1.5	2	0.8	....	....
			0.5	pH	7.0	9.05	9.3	9.22	9.5	....	9.8
				NH <sub>3</sub> -N	0	4	6	5	1.5	....	....
			0.25	pH	7.0	8.85	9.0	8.97	8.6	....	9.2
				NH <sub>3</sub> -N	0	2	6	5	2.5	....	....
Nitrate-N 200 mgm./l.	6	150	1.5	pH	7.0	9.41	9.6	....	9.7	....	10.1
				NH <sub>3</sub> -N	0	0	1	1	0.4	....	....
			1.0	pH	7.0	9.41	9.4	....	9.8	9.9	....
				NH <sub>3</sub> -N	0	1.5	1	1	1.5	....	....
			0.5	pH	7.0	9.14	9.4	....	9.7	....	....
				NH <sub>3</sub> -N	0	4	7	5	2.5	....	....
			0.25	pH	7.0	8.88	9.2	....	9.4	....	9.6
				NH <sub>3</sub> -N	0	3.5	6	6	4	....	....
N <sub>2</sub> -N	10	500	1.5	pH	7.0	9.65	9.6	9.56	9.8	....	9.9
				NH <sub>3</sub> -N	0	0	0.5	0.5	0.7	....	....
			1.0	pH	7.0	9.31	9.5	....	9.6	....	10.1
				NH <sub>3</sub> -N	0	1	1	0.5	0.4	....	....
			0.5	pH	7.0	9.22	9.3	...	9.7	10.0	....
				NH <sub>3</sub> -N	0	3.5	4	1.5	1.5	....	....
Nitrate-N 200 mgm./l.	10	500	1.0	pH	7.0	9.56	9.5	9.59	9.7	10.0	....
				NH <sub>3</sub> -N	0	1.5	1	0.7	0.5	....	....

1-day old, aerated stock *A. vinelandii* culture, growing in 1 per cent glucose medium, placed in cotton-stoppered flasks with volumes as indicated. Temperature, 32°C. Gas phase, air. NH<sub>3</sub>-N (Nesslerization) in mgm./l. Total cell-N (not determined) estimated never to have exceeded 100 mgm./l. pH by quinhydrone electrode (2, 4 days) and colorimetrically (3, 6, 7, 12 days).

Notes: The optimum NH<sub>3</sub>-formation at about 0.5 per cent Na-succinate represented, for the most part, a balance between greater growth but slower completed substrate utilization with increasing succinate concentration. Evaporation of NH<sub>3</sub> from the (cotton-stoppered) flasks played a certain rôle in determining the absolute concentrations of NH<sub>3</sub> observed in the liquid medium. The rapid increase to pH 9 by 2 days is to be noted, and also its continued more gradual rise to a value of 10 after a week or more. The pH change was slightly greater in the larger flasks; the converse was true, however, of NH<sub>3</sub>-formation, probably because of the actual high pH range involved. The pH increases regularly with increasing initial succinate concentration. The cultures obtaining their nitrogen from nitrate gave results in regard to pH change and NH<sub>3</sub>-formation essentially indistinguishable from cultures obtaining their nitrogen for growth from free N<sub>2</sub>. Similar cultures grown in urea, peptone, or asparagin gave NH<sub>3</sub> in the same manner; and also to a certain extent, independently of any growth taking place, by direct deamination of these compounds.

As the predominant new observation, it was established, as an invariable rule, that the formation of  $\text{NH}_3$  never commenced until the concentration of utilizable organic substrate had reached a very low level of about 0.03 to 0.01 per cent (tables 3, 4, 5, 6) and had thus essentially disappeared. It became quite clear that the question of whether appreciable amounts of  $\text{NH}_3\text{-N}$  would appear at all was determined almost wholly by this one factor of concentration of utilizable organic substrate, independently of any other external factor such as pH or temperature varied over a wide range. For various reasons, indicated in table 6, the last traces of glucose disappear very slowly in

TABLE 3

*Influence of residual glucose on  $\text{NH}_3$ -formation by cultures grown with both  $\text{N}_2$  and  $\text{NH}_3$*

	11 DAYS			16 DAYS		
Initial $\text{NH}_3\text{-N}$ , added at 0 days (mgm./l.)	20.0	40.0	60.0	20.0	40.0	60.0
Total-N, mgm./l.	51.2	64.1	78.5	59.7	66.6	84.0
N fixed, mgm./l.	31.2	24.1	18.5	39.7	26.6	24.0
$\text{NH}_3\text{-N}$ , mgm./l.	0.0	0.0	0.0	0.0	0.0	2.3
Glucose conc., per cent.	0.365	0.312	0.276	0.015	0.015	0.000
Cell dry matter (mgm./l.)	468	551	733	551	545	649
N in dry matter, per cent.	10.9	11.6	10.7	10.8	12.2	12.6
pH	7.2	7.2	7.2	7.2	7.2	7.2

100 cc. of 1 per cent glucose medium in cotton-stoppered, stagnant, 1000-cc. (flat) Roux bottles inoculated with 6 drops of 6-day-old, stagnant culture of *A. chroococcum* Strain S.M. 1. Temperature,  $28^\circ\text{C}$ . Gas phase, air. Initial pH  $7.2 \pm 0.1$ . All cultures and analyses performed in duplicate, and averaged.  $\text{NH}_3\text{-N}$  determined by microdistillation (sensitive here to 0.2 mgm./l.), total-N by Kjeldahl, and glucose by Bertrand reducing method (sensitive to 0.002 per cent).

Notes: It is to be seen that by 11 days the glucose was two-thirds consumed, and all added  $\text{NH}_3$  had been utilized, with additional  $\text{N}_2$  fixed, the more so the smaller initial  $\text{NH}_3$ -addition. By 16 days smaller additional amounts of nitrogen had been fixed, and the glucose had in two instances almost but not quite disappeared, whereas in the third instance it had completely disappeared, and there was a definite loss in cell dry matter (presumably through respiration), and  $\text{NH}_3$ -formation had clearly commenced, even at the low pH value of 7.2 involved. The percentage nitrogen in the cell dry matter remained fairly constant in spite of widely varying nitrogen nutrition, and a certain variation in cell dry matter obtained.

stagnant cultures (tables 3, 4, 5, 6) and there is a period of one or more days when  $\text{NH}_3\text{-N}$  may form with small amounts of sugar still present, as in Kostytshev and Scheloumova's experiments (21), as well as in ours. Kostytshev and Scheloumova did not report any analyses of concentrations of residual sugar, and so missed the significance of the concentration factor emphasized here. Indeed, the use of solid media in their experiments prevented in considerable measure any satisfactory determination of substrate concentration effects. In the same way, Winogradsky had observed that  $\text{NH}_3$ -formation commenced, in cultures with alcohol as organic substrate, after disappearance

of the odor of the alcohol, and then rather suddenly; and in cultures containing both mannite and Na-lactate, after browning of the mucus, an indication of disappearing substrate. Thus, although depletion of organic substrate

TABLE 4

*Influence of various initial mixtures of glucose and succinate on  $\text{NH}_3$ -formation*

SUBSTRATE MIXTURE		DAYS											
Succinate	Glucose	1		2			3		4			7	
per cent Na-salt	per cent	pH	NH <sub>3</sub> -N	pH	NH <sub>3</sub> -N	Glucose	pH	NH <sub>3</sub> -N	pH	NH <sub>3</sub> -N	Glucose	pH	NH <sub>3</sub> -N
0	0.1	7.6	0	7.8	0	0.05-0.1	7.7	0	8.1	0	0-0.03	8.3	1.8
	0.2	7.6	0	7.9	0	0.05-0.1	7.7	0.7	8.1	3.5	0-0.03	8.3	3.5
	0.4	7.4	0	7.6	0	0.05-0.1	7.8	0	8.1	1.5	0-0.03	8.1	4.5
	1	7.3	0	7.2	0	0.05-0.1	7.6	3.5	7.9	6.5	0-0.03	7.9	8.5
0.1	0.1	7.6	0	8.5	0	0.05-0.1	8.9	0	8.8	0	0-0.03	8.9	0.8
	0.2	8.0	0	8.4	0	0.05-0.1	8.8	0	8.7	1.5	0-0.03	9.0	1.5
	0.4	7.6	0	8.4	0	0.05-0.1	8.6	2.5	8.8	1.5	0-0.03	8.9	1.8
	1	8.0	0	7.4	0	0.05-0.1	8.5	4.5	8.5	6.5	0-0.03	8.9	3.5
0.3	0.1	8.3	0	8.8	0	0.05-0.1	9.2	0.4	9.2	0.4	0-0.03	9.3	0.3
	0.2	8.4	0	8.6	0	0.05-0.1	9.0	0.3	9.3	0.7	0-0.03	9.4	0.7
	0.4	7.7	0	8.2	0	0.05-0.1	9.1	1.5	9.4	1.5	0-0.03	9.4	1.2
	1	7.7	0	7.7	0	0.05-0.1	8.2	0.8	9.1	5.5	0-0.03	9.3	3.5
0.3 (6 cc. per 150-cc. flask)	0.1	8.4	0	8.8	0	0.05-0.1	9.2	2.2	9.3	1.5	0-0.03	9.1	0.3
	0.2	8.3	0	8.9	0	0.05-0.1	9.3	2.2	9.2	2.5	0-0.03	9.0	1.3
	0.4	8.2	0	8.4	0	0.05-0.1	8.8	0.3	9.2	0.2	0-0.03	9.1	1.5
	1	8.2	0	7.8	0	0.05-0.1	8.5	0	8.7	0	0.05-0.1	9.0	0.7
1	0.1	8.3	0	9.2	0	0.05-0.1	9.6	0	9.7	0	0-0.03	9.9	0
	0.2	8.4	0	9.0	0	0.05-0.1	9.6	0	9.7	0	0.05-0.1	9.8	0.3
	0.4	8.2	0	9.1	0	0.05-0.1	9.6	0	9.6	0	0.05-0.1	9.8	0.3
	1	8.4	0	9.0	0	0.05-0.1	9.5	0	9.6	0	0.05-0.1	9.6	0

500-cc. Erlenmeyer flasks (cotton-stoppered) with 10 cc. of 3-day-old, aerated stock *A. vinelandii* culture diluted fivefold with inorganic medium (pH 8) in order to reduce initial glucose concentration to less than 0.2 per cent. Temperature, 32°C. Gas phase, air.  $\text{NH}_3\text{-N}$  in mgm./l., glucose in per cent.

Notes: The concentrations of  $\text{NH}_3\text{-N}$  formed depend upon concentration and kind of substrate, duration, (unmeasured) growth, and evaporation of  $\text{NH}_3$ . The pH increases regularly with succinate concentration (as in table 2), and decreases with glucose concentration.

was recognized as a factor in  $\text{NH}_3$ -formation, it was not assigned the supreme importance warranted and was considered minor compared to pH and other factors.

Practically no measurements are given, in tables 1 to 8, of the amount of growth involved in any case. It may be said, however, that for  $\text{NH}_3$ -formation the factor of cell mass (cell nitrogen, or cell number, or turbidity) was next in importance to substrate concentration and, in general, of at least equal importance to pH and (where allowed to take place) physical evaporation. Because of the fact that  $\text{NH}_3$ -N never exceeded as a limit about half the cell nitrogen, the amount of  $\text{NH}_3$ -N formed was, in order of magnitude, closely related to

TABLE 5

*$\text{NH}_3$ -content of miscellaneous (not strongly alkaline) stock cultures, in relation to glucose content and age*

AGE	pH	GLUCOSE	$\text{NH}_3$ -N
<i>days</i>		<i>per cent</i>	<i>mgm./l.</i>
54	7.5	0	55
46	7.8	0	55
56	7.2	0	18
56*	7.0	0.01	7
29	7.2	0	6
39	7.3	0.01	5
55*	6.9	0.01	4
15*	6.6	0.01	3
33*	6.6	0.05	0
29*	6.6	0.05	0
39*	6.8	0.08	0
16	7.0	0.05	0
0-10	6.0-8.0	0.05-1.0	0†

Stock *A. vinelandii* cultures grown on 100 cc. 1 to 2 per cent glucose or sucrose medium in 500-cc. gas wash bottles aerated slowly with sterile air.

\* Molybdenum content of medium partially deficient and growth reduced one-half to three-quarters its value with adequate Mo.

† Numerous (*A. vinelandii* or *A. chroococcum*) stock cultures 0 to 10 days old, and still containing 0.05 to 0.10 or more per cent glucose (and varying in pH from 6.0 to 8.0), practically never gave even traces of  $\text{NH}_3$  (less than 0.1 mgm./l.). This was equally true, of course, for many similar cultures up to at least 90 days old.

Notes: The concentration of available glucose completely determines the qualitative appearance of  $\text{NH}_3$ -N; and pH and extent of growth (as varied by age and molybdenum content) are additional, secondary quantitative factors.

the mass of organisms. Any conditions which decreased the extent of growth before the substrate had disappeared, although otherwise favorable to  $\text{NH}_3$ -formation, resulted in smaller eventual yields of  $\text{NH}_3$ .

Tables 1 to 8 illustrate well the complexity and tangling of factors necessarily involved in the sort of technique employed heretofore and in these preliminary studies. In general, satisfactory interpretation of the tables already given requires simultaneous consideration of all the factors indicated in this section; it was, nevertheless, rarely feasible or convenient to carry out all the necessary measurements regarding them. It very soon became evident,

TABLE 6

*Influence of residual glucose concentration and clear centrifuged, mature culture medium on NH<sub>3</sub>-formation*

CULTURE A*	SUSPEN- SION B*	RESIDUE C*	INOR- GANIC MEDIUM*	ADDITIONAL		DAYS				
						2	3	4	6	8
cc.	cc.	cc.	cc.	0.4 per cent						
10					pH	7.4	7.9	8.0	7.9	8.6
					NH <sub>3</sub> -N	0	0	0	0	7.5
					Glucose	...	0.10	0.07	0.04	0.01
	10				pH	7.8	7.9	7.9	8.3	7.8
					NH <sub>3</sub> -N	0	0.5	1.7	27	6.5
					Glucose	...	0	0	0	0
	5	5			pH	7.4	8.0	8.1	8.2	8.3
					NH <sub>3</sub> -N	0	0	0	7.5	14
					Glucose	...	0.1	0.03	0.01	0
	5	2	3		pH	7.7	7.9	7.8	8.3	8.0
					NH <sub>3</sub> -N	0	0	0	11	6.5
					Glucose	...	0.03	0.03	0.01	0
	5	0.5	4.5		pH	7.8	7.7	7.8	8.1	8.0
					NH <sub>3</sub> -N	0	0	0.4	14	2.5
					Glucose	...	0	0	0	0
	5	2	3	Na-suc- cinate	pH	8.5	9.2	9.4	9.5	9.6
					NH <sub>3</sub> -N	0	3.5	4.5	3.5	1
					Glucose	...	0.01	0.01	0.01	0
	5	5		Glucose	pH	7.4	7.6	8.0	8.3	8.3
					NH <sub>3</sub> -N	0	0	0.5	22	17
					Glucose	...	0.05	0.01	0	0

\* 4-day-old, aerated stock *A. vinelandii* culture A centrifuged; clear liquid residue C retained; solid cell residue taken up in 0.1 per cent glucose, inorganic medium at pH 8 at two and a half times original cell concentration in culture A to yield suspension B. These mixed in varying proportions as indicated, using 10 cc. total volume per 500-cc. cotton-stoppered Erlenmeyer flasks. Temperature, 32°C. Gas phase, air. NH<sub>3</sub>-N (Nesslerization) as mgm./l.; glucose as per cent. 100 mgm./l. initial total cell nitrogen in culture A, 250 mgm./l. in suspension B.

Notes: The evaporation of NH<sub>3</sub>-N is to be noted as rather marked between the sixth and eighth day; the rather slow disappearance of the last traces of sugar is probably due to a very low respiration capacity on the part of the cells, and may also be sustained in part by reconversion from small amounts of reserve higher carbohydrate. The low concentration of the sugar also provides for only a small saturation of the respiration enzyme system of the cells. The increased NH<sub>3</sub>-formation due to added glucose is a result of increased cell nitrogen (growth), and this is also true for the case of 10 cc. of suspension B compared to 10 cc. of culture A, where two and a half times as much cell nitrogen (Kjeldahl) is involved. In this experiment the NH<sub>3</sub>-formation has depended chiefly upon available cell nitrogen and residual glucose concentration and scarcely at all upon pH. No clear-cut effect of centrifuged liquid residue, markedly hastening or retarding, is evident. The pH of 9 or more attained with the 0.4 per cent additional succinate has clearly been detrimental.

TABLE 7

*Influence of pH upon total NH<sub>3</sub>-formation and NH<sub>3</sub>-evaporation*

		INITIAL pH..... FINAL pH.....	7.2 7.5	8.0 8.1	8.7 8.4
NH <sub>3</sub> -N	Initial	Nesslerization	0	0	0
	Final	Nesslerization	40	32	23
		Microdistillation	36	32	26
Total-N	Initial	Kjeldahl	125	125	125
	Final		116	...	98
NH <sub>3</sub> -N	Lost	(Evaporation)	9	...	27
NH <sub>3</sub> -N	Total	(Final + Lost)	47	...	52
Total NH <sub>3</sub> -N Initial Total-N, per cent			37	...	42

13-day-old, aerated stock *A. vinelandii* culture centrifuged and taken up in inorganic, borate-phosphate medium at three different pH values, with 6 cc. per 150-cc. cotton-stoppered flask. Temperature, 32°C. Gas phase, air. NH<sub>3</sub>-N and total-N in mgm./l. Duration, 5 days.

Notes: The final amounts of NH<sub>3</sub>-N obtained do not vary greatly, over the pH range studied. Other similar experiments not reported, in which the course of NH<sub>3</sub>-formation was followed, after initial adjustment of pH, indicate an optimum rate at about pH 8, with greatly reduced rates below pH 7 and above pH 9.

TABLE 8

*Rate of evaporation of NH<sub>3</sub> from culture medium, in relation to pH*

	0	1	2	4
pH	8.9	8.2	8.0	7.9
Conc. NH <sub>3</sub> -N (mgm./l.)	10	5.5	3	3
	3	1.5	0.6	0.6
	1.5	0.7	0.5	0.5
	0.5	0.2	0.1	0.1
pH	7.3	7.2	7.2	7.2
Conc. NH <sub>3</sub> -N (mgm./l.)	10	9.5	9.5	9.5
	3	3	2.7	2.7
	1.5	1.5	1.4	1.4
	0.5	0.5	0.5	0.5

10 cc. uninoculated, inorganic (sugar-free) medium in 500-cc. cotton-stoppered Erlenmeyer flasks. Temperature, 32°C. Gas phase, air.

Notes: Up to an initial concentration of 10 mgm./l. NH<sub>3</sub>-N, the factor of evaporation of NH<sub>3</sub> becomes significant only above pH 8. The pH was independent of the NH<sub>3</sub>-N concentration and altered on account of the CO<sub>2</sub> in the air. In other experiments at concentrations of 25 to 50 mgm./l. NH<sub>3</sub>-N evaporation was appreciable below pH 8.



particularly in the case of the three major factors involved—carbohydrate concentration, cell mass, and pH—that no one factor could, by the technique employed, be controlled independently or even made to act uniformly in the same direction. Thus, the use of glucose increased cell mass favorably but tended, unfavorably, to keep the pH too low. Use of organic alkali salts increased the pH favorably up to a certain point, beyond which, however, cell mass, respiration, and  $\text{NH}_3$ -formation became very small; loss by evaporation increased continuously with pH and also with extent of  $\text{NH}_3$ -formation. If cell mass were increased, as by centrifugation, the carbohydrate concentration was more quickly lowered, favorably, but the pH was also lowered, unfavorably, as a result of the increase in respired carbon dioxide. It was, consequently, extremely difficult to obtain reasonably satisfactory functional relationships between  $\text{NH}_3$ -formation and any one independently varied major factor. For example, the optimum pH of about 9 assigned by Winogradsky is only apparent, being, in fact, about a unit too high (fig. 3). The value of 9 was observed in cultures in which the organic alkali substrate had essentially disappeared, either locally or throughout the solid medium, by which time the pH had already, under the particular arrangement of experimental conditions involved, happened to have attained a value actually greater than true optimum; the pH had appeared to be optimum simply because the still more important (insufficiently appreciated) factor of organic substrate concentration was dominating the situation.

#### *Aerobic and anaerobic formation of $\text{NH}_3$ under optimum conditions*

*General technique.* As a result of the preliminary experiments just described, in which it was found that low concentration of organic substrate and large cell mass warranted major attention in observing  $\text{NH}_3$ -formation, it was decided to use centrifuged, washed cultures to provide a large concentration of cells and ready elimination of organic substrate, rather than attempt to obtain the same ends by several days' growth and substrate consumption. Such suspensions could easily be adjusted to the optimum pH of 7.8 to 8.0. With this systematic, more controllable technique it was further desirable and convenient to employ the Warburg micro-respiration apparatus, with its manifold variety of possible experimentation.

It first became necessary to determine whether centrifuging produced any unanticipated effects. All our past experience had provided ~~no~~ support for this possibility when the centrifuged cells were tested in other connections, such as growth, nitrogen fixation, or respiration; and *Azotobacter* cells appeared to be comparatively stable with respect to physical treatment, such as mechanical grinding or drying. This view receives further confirmation in the results presented in table 9. This table indicates that cultures of widely varying age suffer no significant loss of soluble nitrogen compounds due to the processes of centrifuging, washing, and storing (with refrigeration) employed by us. This is true even if distilled water is used instead of inorganic nutrient medium in carrying out the washings. The pH and glucose contents of solution cultures remain essentially unchanged by centrifuging and washing, and the celite used in filtering held back no ammonia and only a small fraction of the soluble nitrogen compounds. It will be recalled also that in table 6 the centrifugate likewise appeared to contain no material noticeably harmful or beneficial to  $\text{NH}_3$ -formation. It is to be noted in table 9 that no  $\text{NH}_3$ -formation took place in the stock cultures 1 to 4 days old, where sugar still remained in considerable quantity, but was commencing in the 10-day culture, where the glucose had become reduced to 0.01 per cent. Even here there was only a trace of soluble nitrogen present. This is in harmony with our entire experience, in which we have rarely if ever observed more than the low order of 0 to 2 per cent of the total cell nitrogen as soluble nitrogen in media surrounding cells and still containing sugar, or maintained for a week at low temperatures in the absence of sugar. In the Warburg vessels, as in the Erlen-

meyer flasks experiments, the pH tends to increase as considerable amounts of  $\text{NH}_3\text{-N}$  form. This is true whether the  $\text{CO}_2$  produced simultaneously is absorbed into alkali or not.

*Extent of  $\text{NH}_3$ -formation.* Tables 9 to 12 and figure 1 present a large number of determinations showing that a remarkably high proportion of  $\text{NH}_3\text{-N}$  is obtainable from *Azotobacter* cells upon spontaneous decomposition. In the

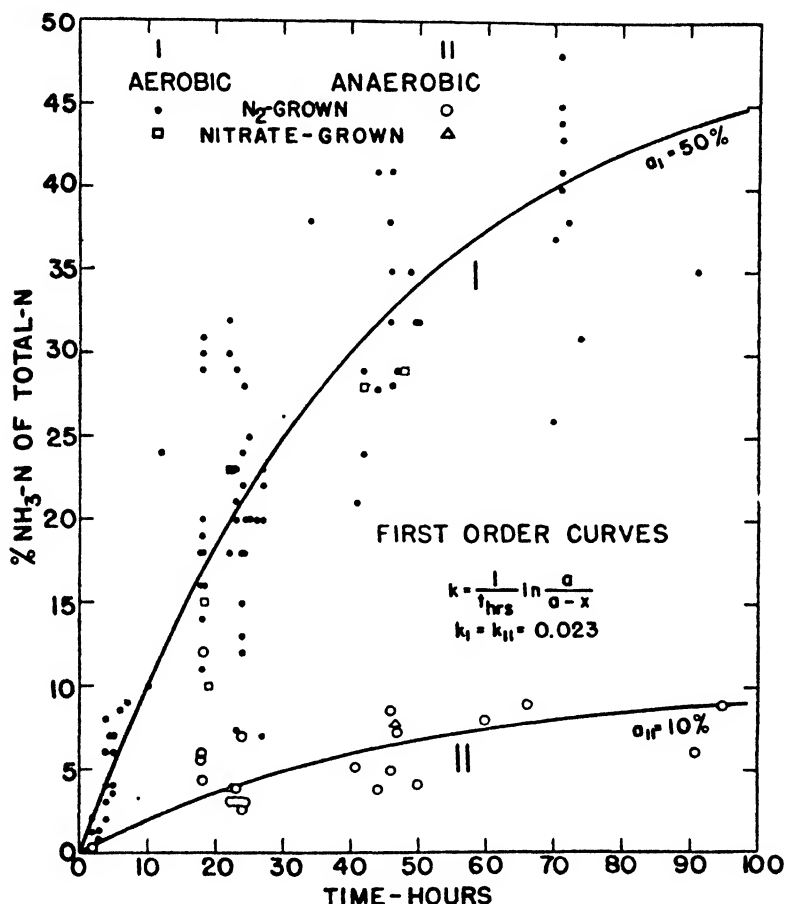


FIG. 1. TIME COURSE OF AEROBIC AND ANAEROBIC FORMATION OF  $\text{NH}_3$  FROM CELLS GROWN IN FREE NITROGEN AND NITRATE NITROGEN

Values taken from tables 9, 10, and 11, based on experiments all conducted in the Warburg apparatus at approximately optimum conditions, namely, with substrate-free medium at pH  $8.0 \pm 0.5$  and at  $31^\circ\text{C}$ .

absence of organic substrate, and under approximately optimum conditions of pH and temperature, a maximum of about 50 per cent of the total cell nitrogen may be transformed to  $\text{NH}_3\text{-N}$  aerobically; and 10 per cent anaerobically, or an extent of 20 per cent as great. About 80 per cent of the transformable nitrogen thus requires external oxidation to form  $\text{NH}_3\text{-N}$ .

TABLE 9

*Influence of age of culture, washing, and centrifuging on cell-nitrogen elution and  $\text{NH}_3$ -formation at different pressures of oxygen*

	AGE (DAYS)					
	1	2	3	4	10	
Stock culture*						
pH	6.7	7.0	6.8	6.8	7.0	
$\text{NH}_3\text{-N}$ , mgm./l.	0	0	0	0	1	
Glucose, per cent	>0.2	>0.2	>0.2	>0.2	0.01	
Filtered centrifugate*						
pH	6.6	6.9	6.8	6.8	6.9	
$\text{NH}_3\text{-N}$ , mgm./l.	0	0	0	0	1	
Glucose, per cent	>0.2	>0.2	>0.2	>0.2	0.01	
Total-N, mgm./l.	0	0	0	0	2.5	
Filtered first and second washings*					(Medium)	Distilled $\text{H}_2\text{O}^*$
pH	7.1	7.0	7.0	7.0	7.0	7.2
$\text{NH}_3\text{-N}$	0	0	0	0	0	0
Glucose, per cent	0	0	0	0	0	0
Total-N, mgm./l.	0	0	0	0	0	0
Stock suspension* (71 hours in Warburg vessels)						
Final pH						
21 per cent $\text{O}_2$ in $\text{N}_2$	8.8	8.6	8.8	8.6	8.5	8.6
21 per cent $\text{O}_2$ in $\text{H}_2$	8.8	8.8	8.8	8.6	8.5	8.6
100 per cent $\text{O}_2$	8.8	8.8	8.7	8.6	.....	8.5
Average	8.8	8.7	8.8	8.6	8.5	8.6
$\text{NH}_3\text{-N}$ , per cent of total-N						
21 per cent $\text{O}_2$ in $\text{N}_2$	43	39	42	45	50	44
21 per cent $\text{O}_2$ in $\text{H}_2$	43	46	42	45	44	44
100 per cent $\text{O}_2$	43	39	35	45	50	44
Average	43	41	40	45	48	44
Total $\text{O}_2$ consumption (cmm./0.1 mgm. cell-N)						
21 per cent $\text{O}_2$ in $\text{N}_2$	285	272	293	267	311	268
21 per cent $\text{O}_2$ in $\text{H}_2$	291	331	336	308	387	351
100 per cent $\text{O}_2$	300	275	351	287	335	316
Average	292	293	327	287	340	312
cc. $\text{O}_2$ per mgm. $\text{NH}_3\text{-N}$ (average)	6.8	7.1	8.2	6.4	7.1	7.1

\* Stock (glucose) cultures grown for progressively shorter periods of time, each older culture serving to inoculate the next younger. After analysis (without filtration) for pH,  $\text{NH}_3\text{-N}$ , and glucose, 80 cc. of each culture (160 cc. of 10-day culture), with 50-300 mgm. cell-N per liter, was centrifuged, under refrigeration below  $30^\circ\text{C}$ . The liquid centrifugate was retained for analysis, and the solid residue washed (30-60 minutes) twice by taking up in 80 cc. inorganic medium (diluted fivefold with distilled water), or in distilled  $\text{H}_2\text{O}$  (0.2 gm./l.

TABLE 9—*Concluded*

phosphate buffer), and centrifuging, this cycle being repeated once again. The clear liquid residues of the washings were combined (for filtration and analysis), and the remaining solid residue was taken up in diluted inorganic medium as a concentrated stock suspension ready for study in the Warburg apparatus. For this purpose they were all diluted to known, approximately the same, concentrations of about 0.06 mgm. cell (micro-Kjeldahl) nitrogen per cubic centimeter. They were diluted with inorganic medium containing 5 gm./l. phosphate buffer at pH 8.0. Each culture was studied in the Warburg apparatus (temperature, 31°C.) in three different gas phases (as indicated), and final  $\text{NH}_3\text{-N}$  and total oxygen consumption were measured. The duration of 71 hours permitted about 90 per cent of complete  $\text{NH}_3$ -formation and oxygen consumption. Before being analyzed, the centrifugate and combined washings were filtered through a thin, well-washed layer of celite to remove the last traces of cells. The celite as used had little or no effect on the chemical compositions of the fluids. pH was determined colorimetrically, glucose and  $\text{NH}_3\text{-N}$  by Nesslerization, and total-N by macro-Kjeldahl (sensitive here to 1.5 mgm./l.).

As indicated in table 9, after prolonged incubation, about 7 cc.  $\text{O}_2$  are consumed per milligram  $\text{NH}_3\text{-N}$  obtained, or 1 cc.  $\text{O}_2$  per 1 mgm. cell dry matter (14 per cent N) initially present. This corresponds to oxidation of protein matter, which requires per unit weight of dry material somewhat less  $\text{O}_2$  than, say, sugar, where 1 cc. oxidizes 1.25 mgm. dry matter. Aerobic  $\text{NH}_3$ -formation is clearly related to oxidation of cell material. A confirmation of this is obtained from the fact that, as will be detailed elsewhere (14), the respiratory quotient ( $\text{CO}_2$  produced/ $\text{O}_2$  consumed) approaches a value of 0.8 to 0.9. In the earlier periods of incubation the R.Q. is at first 10 to 20 per cent greater than unity but gradually falls to the value indicated. No carbon dioxide is formed anaerobically even under prolonged incubation (91 hours).

Dry weight determinations show that the formation of  $\text{NH}_3\text{-N}$  is accompanied by a parallel loss in cell dry matter, and that at complete decomposition, with 50 per cent  $\text{NH}_3\text{-N}$  of total cell-N, the dry matter is reduced to about 50 per cent (table 16).

It is an interesting fact, although not necessarily an unexpected one, that the microscopic appearance of the cells viewed unstained under the microscope is little changed even after loss of 50 per cent of the cell nitrogen as  $\text{NH}_3\text{-N}$ .

*Influence of gas phase.* Hydrogen and nitrogen gases are *completely inert* with respect to rate and amount of  $\text{NH}_3$ -formation and of respiration. The influence of oxygen pressure on cell oxidation is uniform over the range of about 0.01 to 1 atmosphere. This is in marked contrast to the striking oxygen pressure function obtained in respiration with glucose (etc.) as substrate, where  $Q_{\text{O}_2}$  may be some four hundred times as large, 1000 to 5000, and the optimum occurs at 0.15 atmosphere  $\text{O}_2$ , and 30 per cent of optimum rate at, say, 0.01 and 1 atmosphere (5).

*The time course of  $\text{NH}_3$ -formation.* Figure 1 summarizes all the data of tables 9 and 10 on the rate of formation of  $\text{NH}_3\text{-N}$  from *Azotobacter* cells grown in free nitrogen. It is seen that a first order velocity curve with a constant of 0.023 (time in hours) fits both the aerobic and anaerobic course,

TABLE 10  
*Aerobic and anaerobic production of NH<sub>3</sub> from cells of Azotobacter grown in free nitrogen*

STOCK SUSPENSION			N IN DRY MATTER	BILLIONS OF CELLS PER		QO <sub>2</sub>	EXPERI- MENT NO.	STORAGE days†	DURATION hours	PER CENT NH <sub>3</sub> OF TOTAL-N		ANAEROBIC AEROBIC
No.	Species*	Age		Mgm. dry matter	Mgm. N					Aerobic	Anaerobic	
		days	per cent									per cent.
III	chr.-v.	15	—	—	—	—	2	0	27	7	—, 3.0 (N <sub>2</sub> or H <sub>2</sub> )	42
IV	chr.	12	—	—	4.8	9	3	0	3, 23	1.3, 7.3		
V	chr.	‡	15	1.32	7.5	12	4	0	3, 22, 27, 46	0.8, 18, 22, 28		
VI	chr.	‡	8.7	0.65	7.5	16	5	0	2, 22, 70	1.2, 23, 37		
VIII	chr.	19	14.3	0.67	4.7	6	8	1	18, 24, 46	18, 24, 32		
						7	9-10	6	2, 5, 27, 50, 95	2, 3.5, 23, 32	—, —, —, 4.1, 8.7 (N <sub>2</sub> )	13
						7	11	8	18, 46	18, 38		
						—	12	10	18, 46	19, 41		
IX	chr.	5	13.3	0.86	6.5	18	13	0	18, 42	8, 24		
XIII	chr.	13	14.0	0.32	2.3	8	14	0	18	16		
						10	16	2	18	17		
XIV	chr.-v.	9	—	—	4.5	10	17	1	18	20		
						6	18	2	18	30		
						7	19	4	18, 66	29, —	6, 9 (N <sub>2</sub> )	7, —
						8	20	7	4, 18	6, 31	—, 12 (N <sub>2</sub> )	39
						—	22	0	18	11	4.3	39
						12	23	4	5, 23	7, 20		
						10	24	9	5, 24	7, 18		
						—	25	10	24	22		
						16	28	0	24, 44, 91	13, 28, 35	2.5, 3.8, 6.0 (N <sub>2</sub> or H <sub>2</sub> )	19, 15, 17
						16	29-30	5	24, 46	12, —	3, 5 (N <sub>2</sub> )	16, —
						14	31	20	4, 7, 12, 32, 34	3, 9, 24, 30, 38		
						13	32-33	15	4, 23	2, 23	—, 3	13
						13	34	21	10	10		
XV	chr.-v.	14	10.3	1.05	9.8							
XVI	v.	12	19.8	0.76	3.8							

XXIII	v.	21	14.1	0.87	6.4	6	37	5	4, 24 22, 44	4, 32 23, 41	3.9, 5.1 (N <sub>2</sub> ) 0.3, — (N <sub>2</sub> )	17, 13
						—	38	7	2, 25	—, 25	—, —, 8	
						5	44	21	23, 49, 60	29, 35, —	—, —, 8	
						7	46	27	18, 42	14, 29	5.7, —	41, —
						—	47	28	16, 41, 70	16, 38	7, —	
						—	48	32	24, 47	21, 29	—, 7.3	44
XXIV	v.	17	16.7	0.34	2.3	14	49	1	18, 41, 70	16, 38	—, 7.3	—, 26
						14	50	7	24, 47	20, 31		
						18	55	22	27, 74	20, 32		
						14	56	23	25, 50	28		
						18	57	25	24	20, 35		
						16	58	31	26, 46	8	—, 8.7	—, 44
						21	61	48	4	4, 18	—, 3.0	—, 16
XXIX	v.	26	13.8	0.66	4.8	5	64-65	4	5, 24	6, 11, 20		
						—	71	7	5, 25	8, 11		
						—	72	12	6	43**		
XXX	v.	1	—	—	—	(10)	73	1	71	41**		
XXXI	v.	2	—	—	—	(6)	73	1	71	40**		
XXXII	v.	3	—	—	—	(5)	73	1	71	45**		
XXXIII	v.	4	—	—	—	(7)	73	1	71	48**		
XXXIV	v.	10	—	—	—	(7)	73	1	71	44**		
XXXV	v.	10	—	—	—	(6)	73	1	71			
Average.....			13.8	0.75	5.4	10.7						26

Temperature 31°C.; pH 8.0 ±0.5; stock suspensions obtained (as in table 9) by centrifuging air-grown stock cultures and washing twice with (substrate-free) inorganic medium diluted five fold.

\* chr. = *chromococcus*; v. = *vinelandii*; chr.-v. = mixture of cultures of the two species made after growth period and just prior to centrifugation. † QO<sub>2</sub> = cmn. O<sub>2</sub> consumed per mgm. dry matter per hour at start of incubation for NH<sub>4</sub>-formation (usually based on readings for first hour).

‡ Time elapsed from preparation of stock suspension until start of incubation for NH<sub>4</sub> formation, the suspension being maintained meanwhile in the ice-chest at about 5°C.

§ Oxygen-free gas employed, N<sub>2</sub> or H<sub>2</sub> or H<sub>2</sub>, as indicated in parentheses following value.

|| Same value obtained in air, and also in 21 per cent O<sub>2</sub> in H<sub>2</sub>.

¶ Same values obtained in O<sub>2</sub>, air, 21 per cent O<sub>2</sub> in H<sub>2</sub>, 5 per cent O<sub>2</sub> in N<sub>2</sub>, at three different cell concentrations (table 12).

\*\* Same values obtained in air, 21 per cent O<sub>2</sub> in H<sub>2</sub>, and 100 per cent O<sub>2</sub> (table 9); cell dry matter not determined for XXX-XXXV, and QO<sub>2</sub> estimated on basis of 14 per cent N.



XXVI	v.	creatine	16.7	8	59	0	19	45	68		3.6	10	20				
XXVII	v.	alanine	12.7	14	59	0	19	45	68		2.8	8	13				
								114	162					22	26		
			16		60	5	19	43	71		3.6	8.5	14			0.9	1.7
					60a	8	27	49	96							2.5	(—)
					61	13	5			1							20
XXVIII	v.	peptone	12.0	18	59	0	19	45	68		0.4	6	19				15)
			17		60	5	19	43	71		0.4	5.6	22				
								114	162					34	34		
					60a	8	27	49	96	0						1	2
					61	13	5									—	—
Average.....			14.4	15.4							1	5	15	21	28	32	—
											6.6	4	4**	14**			21
											4						21**

Temperature, 31°C.; pH 8.0  $\pm$  0.5; all cultures used in preparing stock suspensions, 5 days old; cultures for X, XI, and XII grown simultaneously, also for XVII-XXII, and for XXV-XXVIII; stock suspensions obtained (as in table 9) by centrifuging stock cultures and washing twice with (substrate-free) inorganic medium diluted fivefold; Qo, determinations averages of three essentially agreeing triplicates, run usually at three different cell concentrations.

\* Chr. = *chromococcum*; v. = *vinelandii*.

† cmn. O<sub>2</sub> consumed per mgm. dry matter per hour at start of incubation for NH<sub>3</sub> formation.

‡ Time elapsed from preparation of stock suspension until start of incubation for NH<sub>3</sub> formation, the suspension being maintained meanwhile in the ice-chest at about 5°C.

§ Columns arranged in order of approximate durations of 5, 18-28, 43-50, 67-71 (and 96), 114, and 162 hours.

|| Cultures for these suspensions grown simultaneously, and closely comparable with, culture for suspension IX, table 10; aerated with a stream of air (compressed); all other cultures of this table grown in O<sub>2</sub>, or a bomb mixture of 50 per cent O<sub>2</sub> in N<sub>2</sub>.

¶ XVIII, XIX, and XX combined after use in experiments 36 and 36a.

\*\* Nitrate-grown cultures excluded.



with only the end points, 50 per cent and 10 per cent  $\text{NH}_3\text{-N}$ , differing. To what extent, if any, aerobic deamination is limited by the anaerobic step, or to what extent it may be considered that the curves indicate a common rate of enzyme decomposition, remains to be determined.

The velocity constant of 0.023 corresponds to an initial rate of decomposition of 1 per cent of the total cell nitrogen per hour, or 2 per cent of the cell nitrogen eventually decomposed. Whereas this rate is only moderate compared with many biochemical and chemical reactions, from the agronomic standpoint it is very rapid and means that a large amount of the nitrogen fixed by *Azotobacter* can be liberated into the soil, almost without significant time limitation, once optimum conditions occur. Thus 50 per cent of the nitrogen available

TABLE 12  
*Influence of different gas phases on  $\text{NH}_3\text{-N}$  formation*

GAS PHASE	FINAL pH	$\text{O}_2$ CONSUMED IN 5 HOURS PER 10 MGM. CELL DRY MATTER	$\text{QO}_2$ CMM. $\text{O}_2$ /MGM./ HR.	$\text{NH}_3\text{-N}$ 5 HOURS
		CMM.		per cent
100 per cent $\text{O}_2$ .....	8.1	278	5.6	8.0
21 per cent $\text{O}_2$ in $\text{N}_2$ .....	8.1	272	5.4	8.0
21 per cent $\text{O}_2$ in $\text{H}_2$ .....	8.1	270	5.4	8.0
5 per cent $\text{O}_2$ in $\text{N}_2$ .....	8.1	254	5.1	8.0
5 per cent $\text{O}_2$ in $\text{H}_2$ .....	8.1	291	5.8	8.0
0.75 per cent $\text{O}_2$ in $\text{N}_2$ .....	7.9	176	3.5	6.0

Stock suspension XXIX, temperature  $35^\circ\text{C}$ ., initial pH 8.0,  $\text{NH}_3\text{-N}$  estimated colorimetrically. Determinations are averages of essentially agreeing triplicates at cell concentrations of 8, 4, and 2 billion per cc. in 1, 1.5, and 2 cc. volumes of culture, respectively, or 12.2, 9.15, and 6.1 mgm. cell dry matter per Warburg vessel (1.69, 1.26, and 0.84 mgm cell-N per vessel). There is no influence on  $\text{QO}_2$  or  $\text{NH}_3\text{-N}$  of either  $\text{H}_2$  or  $\text{N}_2$ , or of varying  $\text{O}_2$  pressures between 100 and 5 per cent, but at 0.75 per cent  $\text{O}_2$ , because of diffusion limitation or otherwise, the  $\text{NH}_3\text{-N}$  is reduced 25 per cent and similarly the respiration about 35 per cent.

for release as  $\text{NH}_3\text{-N}$  (25 per cent of the total cell-N) would be liberated aerobically in 1 day, and 80 per cent within 3 days.

A remarkable feature of the time course is the absence of lag or induction. When suitable optimum conditions are provided the process of decomposition sets in immediately in a manner that can be characterized by usual and simple physicochemical treatment. This point is highly significant in connection with the nature and general certainty of the process.

The rather large spread of points in figure 1 is due partly to small but definite deviations from strictly optimum conditions (pH variation of 1 unit, etc.), and also to aliquoting and to accumulative errors in  $\text{NH}_3\text{-N}$  and total-N determinations. The points represent chiefly the controls in some 70 different experiments, using 20 different stock suspensions, and carried out over a period of a year; from this point of view the spread is in reality remarkably small, rather than large as might appear at first glance. It will be seen, for instance, that although

the average  $Q_{O_2}$  is 15.4 (table 10), there is a variation, among the stock suspensions, of from 5 to 20; and similarly the average "billions of cells per milligram of dry matter," 0.75, varies from 0.34 to 1.32; and the average "billions of cells per milligram of cell-N," 5.4, varies from 2.3 to 9.8. This variation in the nature of the cells of different stock suspensions is large or small, depending upon one's point of view. Although the nitrogen content of the dry matter of the cells was usually close to 14 per cent, a variation from 8.7 to 19 per cent was observed. In this latter connection, as is well known, cultures grown on agar usually have much lower nitrogen contents of 2 to 5 per cent (11), due merely to incidentally accompanying carbohydrate or gum. With any one stock suspension duplication of results was of course usually very satisfactory (tables 10, 11). Also, very carefully conducted individual experiments gave first order (logarithmic) curves in the same manner as the grand average of figure 1.

The age of cultures maintained in organic substrate, varied up to 30 days, or the period of storage of suspensions maintained at low temperature and without organic substrate, varied up to 60 days, had practically no influence on the rate or extent of  $NH_3$ -formation or cell oxidation. This provides strong incidental support for the straightforward physicochemical nature of the process indicated in figure 1 and shows that *Azotobacter* cells are at all times ready to yield  $NH_3$  under proper conditions. Still further support for this view may be drawn from the fact that cell concentration was without consistent influence upon either the rate or the extent of ammonia formation per unit amount of cells. In this connection the concentration was varied from 0.1 to 20 billions per cubic centimeter. There is a direct relation between yield of  $NH_3$ -N and cell mass or cell-N; *the cells thus act as sources of  $NH_3$ -N and not essentially as catalysts for direct production of  $NH_3$ -N from some other source, such as  $N_2$  and glucose.*

In the same way it has been impossible to detect a statistically significant difference in regard to  $NH_3$ -formation and related phenomena between the strains of *A. chroococcum* and *A. vinelandii* used. The two species types (11, 17, 26) could, nevertheless, be clearly distinguished upon other grounds; they maintained consistently, even after several years' cultivation on laboratory media, the different pigment formations necessary for their differentiation. *A. chroococcum*, black pigmentation, *A. vinelandii*, green or pink coloration (17). Green (11) found *A. vinelandii* to contain a considerably lower percentage carbohydrate and higher percentage of nitrogen than *A. chroococcum*, but fractionation of the nitrogen showed no essential differentiation.

As other incidental factors, it may be mentioned that, in general, shaking the incubating suspensions, as in the Warburg apparatus, was without influence so long as diffusion limitation of oxygen was avoided. Except for any minor changes in pH involved, the rate of  $NH_3$ -formation was independent of the presence of ( $CO_2$ -absorbing) alkali in the wells of the Warburg vessels and also of  $H_2SO_4$  placed in the side-arms. The  $H_2SO_4$  would not usually absorb the  $NH_3$  as fast as it was formed in the culture in the main part of the vessel, and so could not be used with great advantage in measuring the  $NH_3$ -N formed.

*Cells grown in fixed nitrogen.* Table 11 shows that, in principle, and for the

most part in detail also, cells grown in widely varying forms of fixed nitrogen yield  $\text{NH}_3\text{-N}$  in the same manner as cells grown in free nitrogen. Aerobically and anaerobically, limits of 50 and 10 per cent of total cell-N are obtained as  $\text{NH}_3\text{-N}$ , after sufficient incubation. The nitrogen content in the dry matter averages 14.4 per cent (instead of 13.8 in table 10).  $Q_{\text{O}_2}$  averages 15 instead of 11 (probably not significantly different, statistically).

The only definite distinction noticeable is in regard to a slower rate of  $\text{NH}_3\text{-N}$  formation with *certain* of the nitrogen compounds. Nitrate-N gave cells indistinguishable from those grown in free  $\text{N}_2\text{-N}$  (fig. 1 and table 2). However, it would appear that compared to the nitrogen sources,  $\text{N}_2$  and nitrate, the other nitrogen compounds gave suspensions which yielded  $\text{NH}_3\text{-N}$  at an initial rate, for the first 10 to 20 hours, about one third as great, as a grand average. As a matter of fact, however, the entire series may be arranged in order of rate of formation of  $\text{NH}_3\text{-N}$ , *inversely* as the following approximate order of the rates of growth of the particular original stock cultures from which the stock suspensions were derived, thus: group 1, peptone, ammonia, urea, and glutamate; group 2, alanine, and creatine; group 3, asparagine and adenine; group 4, nitrate and  $\text{N}_2$ . This is true both for aerobic and for anaerobic decomposition. Many other phenomena of *Azotobacter* cell physiology show this inverse relationship with rate of growth; in this connection, for example, may be cited without further discussion the stimulation by soil humic acid, the effect of temperature upon stimulation by soil humic acid, the toxic effect of high oxygen pressures, and the rate of respiration in the presence of glucose.

The following values of "per cent  $\text{NH}_3\text{-N}$  of total-N in first 24 hours" will give some idea of the approximate relative initial rates under aerobic conditions: groups 1, 2, 3, 4: 0.5 to 2, 3 to 5, 6 to 10, 15 to 25 per cent, respectively. With groups 1 and 2 (and to some extent 3) there is a definite induction period, and the rate-time curve is concave upward at first, instead of concave downward (logarithmic) as in figure 1 for group 4. After the lag period the specific velocity constants approach but never exceed the value for group 4 (of about 0.02). Upon repetition of experiments with given nitrogen sources, the arrangement of the four groups would appear to be reasonably consistently maintained; but incidental growth factors not controlled entirely (rate of aeration, duration of growth, size of inoculum, etc.) no doubt also played a minor rôle in the particular results reported in table 11.

It is possible to interpret the inverse relation discovered, upon the *ad hoc* view that the faster rate of growth indicated a more ready utilization of a given nitrogen source and hence a greater opportunity to build the nitrogen into somewhat more complex, less easily broken down compounds. Since, however, the final amounts of  $\text{NH}_3\text{-N}$  formed are independent of nitrogen source, it is much more probable that the enzyme systems concerned in breakdown are not equally developed, a physiological phenomenon now recognized to be of very common occurrence. This latter view is supported by the fact that, in general, when rates of  $\text{NH}_3\text{-N}$  formation 30 per cent suboptimal or less are involved, as, for instance, a function of pH or temperature, the time curves are likewise of the concave upward type involving more or less induction or lag.

In any case, it is evident that, as might be expected, although there are significant differences detectable between cultures grown in different nitrogen sources, they are of an order not so much greater than differences between cultures grown at different times on the same nitrogen compound. For all practical purposes, then,  $\text{NH}_3$ -formation by cells grown in free and in various forms of fixed nitrogen must be regarded as essentially the same process, as far as observed. In view of the large  $\text{NH}_3$ -formation involved this result obviously has a very pertinent bearing on the question of the origin of any  $\text{NH}_3$ -N obtained from cultures grown in free  $\text{N}_2$  and strongly suggests that all of the  $\text{NH}_3$ -N so obtained is derived immediately from cell nitrogen and only remotely, from a mechanistic point of view, from  $\text{N}_2$ .

#### *The inhibition of $\text{NH}_3$ -formation by organic substrate*

In this section will be shown in a very decisive manner, in several different ways, the striking inhibition of  $\text{NH}_3$ -formation by oxidizable organic substrate. In all cases it will be evident that the mechanism of inhibition operates through inhibition of the oxidation of the cell material itself, which, as indicated earlier, is intimately tied up with aerobic  $\text{NH}_3$ -formation.

*Concentration function.* It was found with the Warburg technique that, just as in the Erlenmeyer flask technique, no  $\text{NH}_3$  formed until the oxidizable substrate had decreased to a certain very low value which was ascertained not only chemically but also by calculations based on the observed total oxygen consumption. The same limiting, low value of about 0.01 to 0.03 per cent substrate was found. It was also found that this value could be interpreted in terms of respiration rate. Thus, in general, inhibition of  $\text{NH}_3$ -formation became easily detectable just as soon as the organic substrate increased the rate of oxygen consumption by 25 per cent or more over that given by the cell material itself. The functions involved are clearly demonstrated in figure 2, where reciprocal relations between  $\text{NH}_3$ -formation and respiration rate *in the presence of substrate* are shown to occur over the same concentration range, 0.01 to 0.03 per cent, the same range, in fact, that was observed in the Erlenmeyer flask technique. Glucose and succinate affected the two processes identically. With organic substrates which are oxidized less readily than these, the concentration range would be shifted higher for both processes. In other words, the actual concentration range providing inhibition of  $\text{NH}_3$ -formation is significant insofar as it is the concentration range which significantly increases the cell respiration. Evidence will be provided elsewhere (14), in connection with studies on the respiratory quotient, that the organic substrate is competitively oxidized in preference to cell material, and that the oxidation of the latter (along with  $\text{NH}_3$ -formation) is partially or completely suppressed.

*Substrate groupings.* Table 13 shows, for many different organic substrates, the same sort of inhibition as that obtained in figure 2 with succinate and glucose. Here the quantitative relations are not demonstrated with quite the precision of figure 2, since the respiration rates are given for the first and eighteenth hours only. It will be seen (in XIII-14, XIII-16, XIV-17, or IV-3) that wherever the rate of oxygen consumption is increased at either of these periods,  $\text{NH}_3$ -formation is either partially or completely inhibited. In some cases, at low concentrations, respiration increase is observed at the first hour

but not at the eighteenth hour, because the organic substrate had by this time been completely consumed. In other cases marked respiration increase was noted at the eighteenth hour but none at the first hour because of a lag of several hours involved with some organic substrates before they are utilized. These lag periods have been studied and portrayed in detail by Lineweaver (25). Respiration rate values are given in table 13 for two standardized, significant periods of time only; namely, when  $\text{NH}_3$ -formation was just commencing, and

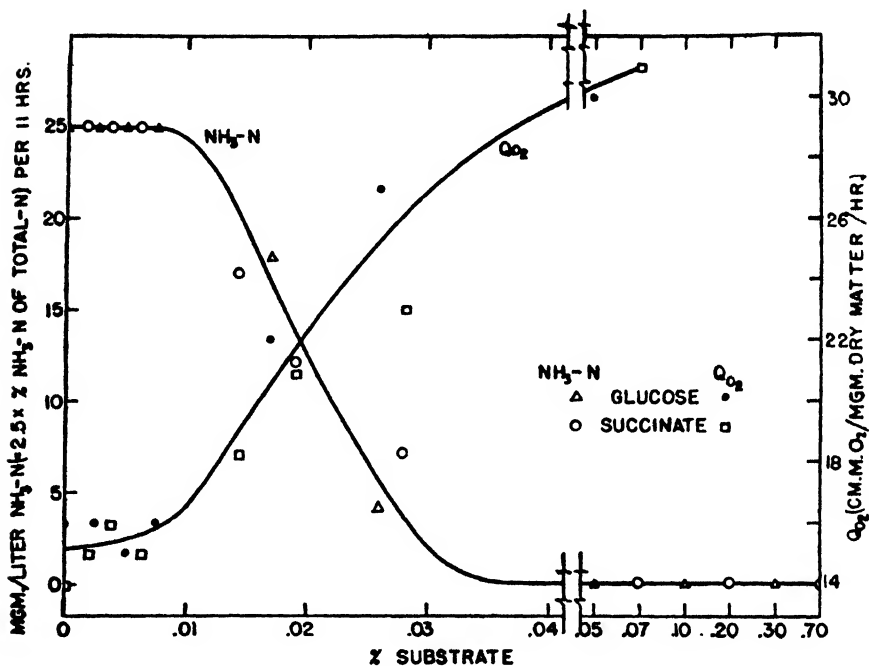


FIG. 2. INFLUENCE OF CONCENTRATION OF SUBSTRATE UPON RATE OF  $\text{NH}_3$ -FORMATION AND RATE OF RESPIRATION

1.5 cc. per Warburg vessel of stock suspension XVI (table 10) diluted to contain 250 mgm./l. cell nitrogen, and maintained at pH 8 and  $31^\circ\text{C}$ . Duration of experiment, 11 hours;  $Q_{O_2}$  given for eleventh hour. Substrate concentrations corrected for slight decreases due to substrate consumption (above 0.01 per cent, 15 per cent correction at most, on a time average). Succinate refers here to succinate ion (M.W. 116), not to sodium succinate (M.W. 162) as otherwise usual.

when 40 to 60 per cent completed, but in these and most of our other experiments measurements were taken periodically throughout the whole time course.

It may be said in regard to oxidation of cell material in the absence of substrate that, in general, the rate of oxygen consumption or, better, the total oxygen consumption, followed the same rough time course as  $\text{NH}_3$ -formation shown in figure 1. For instance, in one particular experiment, out of over

TABLE 13

*Influence of added organic substrates on respiration and on aerobic and anaerobic NH<sub>3</sub>-formation in stock suspensions*

GAS	STOCK SUSPENSION	SUBSTRATE	CONCENTRATION	pH	O <sub>2</sub>			NH <sub>3</sub> -N, 18TH HOUR		PER CENT CHANGE, 18TH HOUR	
	No. and experiment		per cent	18th hour	1st hour	18th hour	mgm./l.	Per cent of total-N	CO <sub>2</sub> (in- crease)*	NH <sub>3</sub> -N (inhib.)	
Air	XIII-14	.....	.....	8.6	8	8	65	15	.....	.....	
		Sucrose†	0.01	8.6	8	8	65	15	0	0	
			1.0	7.6	10	62	0	0	620 (125)	100	
		l-arabinose†	0.001	8.7	8	8	65	15	0	0	
			0.1	8.7	8	8	65	15	0	0	
			1.0	8.4	8	8	65	15	0	0	
		Maltose†	0.01	8.7	8	8	65	15	0	0	
			1.0	7.0	12	86	0	0	720 (150)	100	
Air	XIII-16	.....	.....	8.7	12	10	75	17	.....	.....	
		Levulose†	0.05	8.6	35	10	75	17	..... (280)	0	
			0.2	8.0	41	10	4	0.9	..... (350)	85	
			1.0	7.7	62	53	0	0	530 (520)	100	
		Na-succinate†	0.05	9.1	20	10	65	15	..... (160)	8	
			0.2	9.3	26	24	18	4	240 (240)	75	
			1.0	9.2	39	77	0	0	770 (330)	100	
		Na-acetate†	0.05	8.9	11	14	75	17	140	0	
			0.2	9.2	12	18	65	15	180	8	
			1.0	9.2	20	105	0	0	1050 (160)	100	
Air	XIV-17	.....	.....	8.5	6	6	90	20	.....	.....	
		Na-glycerophos- phate†	0.05	8.6	6	6	90	20	0	0	
			0.2	8.6	6	6	90	20	0	0	
			1.0	8.0	8	6	75	17	0 (125)	15	
		Na-benzoate†	0.05	8.7	6	6	75	17	0	15	
			0.2	8.6	6	9	35	8	150	40	
			1.0	8.1	6	46	0	0	760	100	
Air	XXIII-37	.....	.....	8.3	..	17	104	32	.....	.....	
		Na-maleate§	3.2	8.2	..	0.3	23	22	—98	78	
		Na-oxalate§	1.34	8.2	..	7	65	21	—62	38	
			2.7	8.8	..	1	18	17	—95	83	
		Na-glycollate§	1.0	8.3	..	3	45	43	—83	57	
			10.0	8.2	..	0	13	12	—100	88	
		Na-formate§	0.7	9.0	..	4	35	34	—76	66	

QO<sub>2</sub> as cmm. O<sub>2</sub>/mgm. cell dry matter/hour, NH<sub>3</sub>-N determined by Nesslerization, pH values colorimetric. Initial pH 8.4. Temperature 31°C. In experiments 7 and 3, the duration was not 18 hours as indicated, but 72 and 25 hours respectively.

\* Values in parenthesis indicate per cent increase in QO<sub>2</sub>, if any, at first hour.

† Substrates oxidized and inhibiting NH<sub>3</sub>-formation.

‡ Substrates not oxidized and not inhibiting NH<sub>3</sub>-formation.

§ Substrates not oxidized and inhibiting NH<sub>3</sub>-formation.

TABLE 13—*Concluded*

GAS	STOCK SUSPENSION	SUBSTRATE	CONCENTRATION	pH	QO <sub>2</sub>		NH <sub>3</sub> -N, 18TH HOUR		PER CENT CHANGE, 18TH HOUR	
	No. and experiment		<i>per cent</i>	18th hour	1st hour	18th hour	<i>mgm./l.</i>	Per cent of total-N	QO <sub>2</sub> (in- crease)*	NH <sub>3</sub> -N (inhib.)
Air	IV-3	.....	.....	7.8	9	9	100	7.3	.....	.....
N <sub>2</sub> or (H <sub>2</sub> )	IV-3	Glucose†	1.0	7.5	32	105	0	0	1150 (350)	100
		.....	.....	7.4	..	.....	40	3.0	.....	.....
		Na-succinate	1.0	7.4	..	.....	40	3.0	.....	0
		Glucose	1.0	7.4	..	.....	40	3.0	.....	0
N <sub>2</sub>	VIII-7	.....	.....	7.2	..	.....	35	3.5	.....	.....
N <sub>2</sub>	XVI-33	Na-formate	0.5	7.4	..	.....	35	3.5	.....	0
		.....	.....	7.8	..	.....	30	3.0	.....	.....
		Glucose	1.0	7.8	..	.....	30	3.0	.....	0

|| These two experiments (aerobic and anaerobic) performed simultaneously.

five hundred, the rates of oxygen consumption at 1, 44, and 91 hours were 80, 20, and 8 cmm. O<sub>2</sub>/vessel/hour, respectively (Stock Suspension XVI). The same correspondence between courses of respiration and of NH<sub>3</sub>-formation was also observed with fixed nitrogen-grown cultures, even where induction and several fold longer incubations were involved. In the cases of a very few cultures the rate was occasionally observed to increase for the first 24 hours, as though some less readily available stored cell substance, such as gum, was being broken down into a more readily available material.

Table 13 presents some very interesting qualitative groupings of organic substrates. There are substrates, like glucose or succinate, which are oxidized and inhibit NH<sub>3</sub>-formation in the manner already described. There are also substrates (arabinose, glycerophosphate) which are not appreciably oxidized, and which, in accordance with our now developed, general understanding and theory should not, and do not, inhibit NH<sub>3</sub>-formation. A third group (maleate, oxalate, glycollate, and formate) are not appreciably oxidized, but independently, at comparatively very high concentrations, inhibit both NH<sub>3</sub>-formation and oxidation of cell material. Finally, under the imposed anaerobic conditions, where oxidation of both substrate and cell material is prevented, substrate inhibition is in no instance observed. (This is particularly interesting in the case of those substrates shown which, under aerobic conditions, are oxidized and inhibit NH<sub>3</sub>-formation.) These four qualitative groupings show independently, in a very striking and effective manner, the important parallelism, a relation of cause and effect, between oxidation of cell substance and formation of NH<sub>3</sub>-N.

The fact that oxidation of organic substrate completely inhibits NH<sub>3</sub>-formation aerobically, shows that anaerobic NH<sub>3</sub>-formation has to be understood as more than simple hydroly-

sis, otherwise the underlying process should occur under aerobic conditions also and one should thus obtain a yield of some 20 per cent of the transformable nitrogen [10 per cent of the cell-N (fig. 1)]. Certain oxidation-reduction relations may be involved, as is now known to be the case with some deaminases (e.g., urease, arginase); or some oxidation intermediate may inhibit the process analogously to  $O_2$  in the Pasteur-Meyerhof reaction (aerobic inhibition of fermentation).

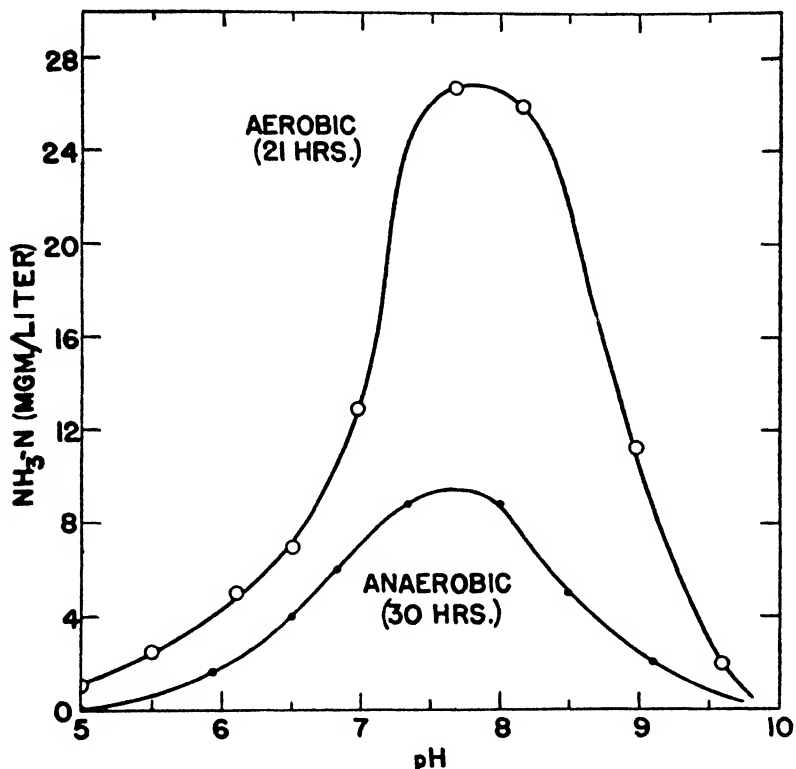


FIG. 3. INFLUENCE OF pH UPON THE AEROBIC AND ANAEROBIC FORMATION OF  $NH_3$

1.5 cc. per Warburg vessel of stock suspension XVI (table 10) diluted with inorganic (substrate-free) medium, containing 10 gm. phosphate mixture, to 260 mgm./l. cell nitrogen, and maintained at 31°C. Anaerobic cultures maintained in  $O_2$ -free  $N_2$ . Initial and final pH values essentially identical.

#### *Secondary and incidental factors in $NH_3$ -formation*

*pH.* Figure 3 presents the pH functions obtained for aerobic and anaerobic  $NH_3$ -formation when all other factors are held essentially at optimum and the organic substrate, in particular, is eliminated from the medium. The optimum pH is seen to occur at 7.8 to 8.0, with half maximal values at 6.5 to 7.0 and 8.5 to 9.0; the anaerobic curve is possibly shifted to a slightly more acid range than the aerobic. Of especial interest, too, is the ammonification measurable even below pH 5.0 and above 9.5, particularly upon more prolonged incubation than



that involved in figure 3. For all practical purposes  $\text{NH}_3$ -formation occurs over a very wide physiological range, and is not limited to a markedly alkaline range, as believed by Winogradsky, or to a mildly acid range, as supposed by Isakova (15). With less controlled technique, as with Erlenmeyer flasks, or

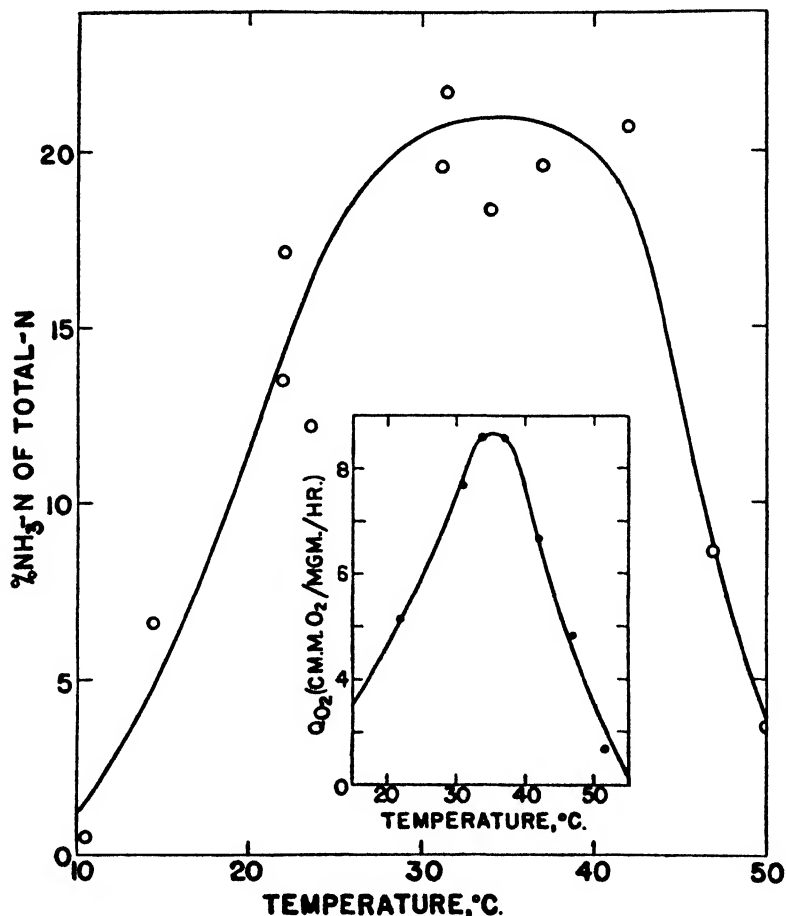


FIG. 4. INFLUENCE OF TEMPERATURE UPON THE RATE OF  $\text{NH}_3$ -FORMATION AND THE RATE OF RESPIRATION

1 cc. per Warburg vessel of stock suspension XV (table 10) diluted with organic substrate-free medium to contain 1020 mgm./l. cell nitrogen, and maintained at pH 8. Duration of experiment, 24 hours.  $Q_{O_2}$ , the rate of respiration in cmm.  $\text{O}_2$ /mgm. cell dry matter/hour, is based on the average rate for the first 5 hours.

with silica gel media, and with evaporation permitted, we ourselves have been able to observe apparent optima varying from 6.5 to 9.5, due to the operation of comparatively incidental factors. The great majority of deaminases have an optimum activity in the region of pH 8, the same as that found in figure 3.

Although the rate of  $\text{NH}_3$ -formation is appreciably reduced at pH values greater or less than 7.5 to 8.5, the final amount of  $\text{NH}_3$ -N produced after somewhat more prolonged incubation is independent of pH, except at the very extreme limits where the rate has been reduced to 10 to 20 per cent or less of the maximum and where induction or irreversible inactivation may occur. Because of this, the rate curves presented in figure 3, particularly the aerobic curve, are possibly a little steeper than curves obtained by measurements not involving a time integration over a number of hours. In any case, the rates remained reasonably constant over the time period required for their measurement, at all except the very limiting pH values.

The curve for rate of oxidation of cell material as a function of pH is similar to that for  $\text{NH}_3$ -formation, being perhaps a little narrower.

*Temperature.* Figure 4 shows that, as with pH, the temperature function is very broad, with an optimum between 30° to 40°C., half-maximal values at 20° and 46° to 48°, and limits at 5° and 55°C. or beyond. At low temperatures the same phenomenon of lag (and steepening of curve) is observable as in the case of very low pH values or as with certain slowly decomposing, fixed nitrogen-grown, suspensions described earlier. Thus, in the case of one stock suspension maintained at about 8°C., the following concentrations of  $\text{NH}_3$ -N were observed to accumulate: 0.3, 0.7, 3.1, 4.6, 9.0, 16 per cent of total-N, after 4, 6, 9, 14, 23, 36 days, respectively. Stock suspensions maintained for a week or more at 0° to 2°C. will show a prolonged lag of many hours upon being incubated at optimum temperature, but after eventual recovery the final amounts of  $\text{NH}_3$  formed and cell oxidation are normal.

The curve for respiration rate as a function of temperature (inset, fig. 4) is similar to, although somewhat narrower than, that for  $\text{NH}_3$ -N. This is true both for rates established without lag and for those at the limits of the function involving induction and again indicates a close relationship between the two processes.

*Buffers and salts.* At constant, approximately optimum pH (7.5 to 8.5), potassium phosphate mixture gives no indication of toxicity when used up to at least 10 gm./l. Borate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ), which gives the same pH function for both  $\text{NH}_3$ -formation and cell oxidation as phosphate, inhibits the rates of both these processes 25 to 75 per cent, at a concentration of only 0.5 to 1 gm./l. The total  $\text{NH}_3$ -N formed after an extended period of time is not affected, except possibly at 5 to 10 gm./l. of borate. The borate inhibition of rate is not due to phosphate absence because it may also be obtained in a mixture of 5 to 10 gm./l. borate and 1 gm./l. phosphate. Increasing the proportion of phosphate will overcome borate toxicity, however. The rate of  $\text{NH}_3$ -formation is not greatly affected by the absence of salts in the medium, but proceeds normally in a suspension made up with distilled water and buffered at pH 8 with a small amount of phosphate mixture.

*Pasteurization.* 15 minutes' pasteurization at 55°C., or 5 minutes' at 75°C. or 95°C., completely inhibits  $\text{NH}_3$ -formation and cell oxidation for a few hours, but by 10 to 15 hours both processes are restored to almost the initial rate, and the final extent of  $\text{NH}_3$ -formation and oxidation is unaffected. This is also true even for 30 minutes' treatment at 95°C. The essentially non-vital (strictly enzymic) nature of the processes is thus strongly indicated. A more detailed study of the aspects of recovery would undoubtedly be of considerable interest.

*Toluene.* A saturated solution of toluene inhibits to the extent of 80 to 90 per cent  $\text{NH}_3$ -formation and oxidation of *Azotobacter* cells and also, as will be shown elsewhere (14), to varying degrees the same processes carried out by cells acting upon fixed nitrogen compounds such as aspartate, arginine, guanine, allantoin. In striking contrast, however, the anaerobic formation of  $\text{NH}_3$  is entirely unaffected by toluene. Kostytshev and Scheloumova (21) had observed considerable aerobic inhibition of ammonification (and also of fixation). Winoogradsky (34) found toluene did not stop  $\text{NH}_3$ -formation completely. Toluene evidently does not greatly prevent the anaerobic (hydrolytic) process from taking place when  $\text{O}_2$  is present, in marked contrast to the action of oxidizable organic substrate, where the inhibition of  $\text{NH}_3$ -formation may be definitely complete.

*Cyanide.*  $\text{NH}_3$ -formation and cell respiration are affected in an identical manner by cyanide, 50 per cent inhibition being obtained at about  $10^{-3} M$ . The same is also true with alanine as a substrate, except for greater sensitivity, 50 per cent inhibition occurring at  $10^{-4} M$ . According to Lineweaver (25), 50 per cent inhibition of glucose oxidation by cells occurs at a still lower concentration,  $5 \times 10^{-4} M$ , whereas anaerobic dehydrogenation of glucose by methylene blue in the presence of *Azotobacter* cells is not affected at all at even  $2 \times 10^{-3} M$ . The effect of cyanide on anaerobic  $\text{NH}_3$ -formation from *Azotobacter* cells has been determined to be extremely weak (14). In making the aerobic determinations it was necessary to estimate the respiration in the absence of  $\text{CO}_2$ -absorbing alkali, otherwise the cyanide would likewise be absorbed completely from the medium in a short time. The experiments with cyanide will be detailed elsewhere (14) in connection with additional experiments with added fixed nitrogen substrates capable of being ammonified.

*Nitrate.* Kostytshev considered that *Azotobacter* reduced nitrates directly to  $\text{NH}_3$ , in the same manner as he held  $\text{N}_2$  to be so reduced, and also in the same manner as he and Tzwetkova (24) and Klein, Eigner, and Müller (16) had observed fungi, and Warburg and Negelein (31) had observed the green alga *Chlorella*, to do. However, for the same reasons as those involved in the case of  $\text{N}_2$ , it is impossible to determine from Kostytshev's experiments whether with *Azotobacter* the  $\text{NH}_3$  observed was really derived directly from nitrate or indirectly through formation of normal cell nitrogen.

Using stock suspensions, we have found under aerobic conditions no trace of influence of nitrate on  $\text{NH}_3$ -formation, either in the presence or absence of glucose or other organic substrate. The nitrate had, in fact, no effect on  $\text{NH}_3$ -formation from cell-N except at very high concentrations of 1000–2000 mgm./l. nitrate-N or more.  $\text{NH}_3$ -formation and accompanying cell oxidation are then inhibited. The possibility of direct reduction of nitrate to ammonia by *Azotobacter* cannot be ruled out, of course, but any previous demonstration is very questionable.

Under strictly anaerobic conditions, not considered by other workers heretofore, the situation is quite different. Here, in the absence of sugar, nitrate reacts as an oxidizing agent with cell material and forms, at concentrations ranging from 5 to 2000 mgm./l. N, increased amounts of ammonia as compared with those obtained in the absence of the nitrate. In the presence of glucose and nitrate, as we have shown (4), growth may take place anaerobically with, incidentally, the same pH function. Under these conditions, the nitrate consumed is partially utilized in growth, so that the net result of adding glucose to anaerobic cell suspensions containing nitrate is usually to decrease the yield of  $\text{NH}_3$ -N obtained.

The anaerobic production of  $\text{NH}_3$  by cells in the presence of nitrate and in

the absence or presence of organic substrate is accompanied, as might be expected, by the formation of carbon dioxide, since the nitrate is providing bound oxygen to oxidize cell material (or substrate) in a manner analogous to  $O_2$  gas under aerobic conditions. It will be recalled that in anaerobic  $NH_3$ -formation by cells, in the absence of nitrate, no carbon dioxide is produced.

The relations obtained with nitrate are apparently given, as might also be expected, with nitrite, but to a much less noticeable extent. Under strictly anaerobic conditions nitrite is actually formed from nitrate, as will be considered later (14).

#### *Ammonification of fixed nitrogen substrates*

For completeness of perspective, brief mention will be made of results to be detailed elsewhere (14) which show that *Azotobacter* suspensions are readily able to ammonify, partially or completely, a large number of nitrogenous compounds, including proteins, amino acids, nucleic acids, and simple and substituted amides, in much the same manner, and with much the same rate, as *Azotobacter* liberates ammonia from its own cell nitrogen. Anaerobic ammonification is somewhat more limited than aerobic, affects only a few amino groups, and is confined chiefly to various amide compounds, which are seldom ammonified completely, particularly in the case of ring compounds. A number of compounds, including certain higher amides, simple amines, substituted ureas, and urethanes, are not ammonified either aerobically or anaerobically and are not even oxidized. In general, oxidizable, non-nitrogenous substrates inhibit aerobic ammonification of nitrogenous substrates in the same variety of ways as they do ammonification of cell nitrogen and, likewise, they have little or no effect under anaerobic condition. The relations with respect to gas phase, pH, and narcotics such as toluene and cyanide are also similar. These and many other unmentioned findings with respect to fixed nitrogen substrates for ammonification all lend much support to conceptions put forward in this paper, and will be shown to provide a close insight into the more strictly chemical aspects of  $NH_3$ -formation from cells (14).

#### DISCUSSION

##### *Quantitative critique*

The many and varied results presented here oppose unanimously schemes A and B described in the Introduction, according to which the extracellular ammonia *observed heretofore* is derived wholly or in part by *direct* synthesis from  $N_2$ . This statement is made upon the basis of the following new findings demonstrated in this paper: the non-simultaneity of  $N_2$ -disappearance and  $NH_3$ -appearance; the similar formation of  $NH_3$  by cultures grown in fixed nitrogen and in free nitrogen; the extensive ammonifying capacity of *Azotobacter* cells acting upon various fixed nitrogen substrates; the close quantitative correlation between  $NH_3$ -formation and oxidation of *Azotobacter* cell material, demonstrated under a great variety of circumstances with respect to amount,

rate, and respiratory quotient; and the quantitative limit for the maximum amount of  $\text{NH}_3$  formed in relation to cell material. In this last connection the following analysis of previous investigations used in support of schemes A and B may be made, concluding that given in the Introduction.

The ease and delicacy with which rather small concentrations of  $\text{NH}_3$  could be measured and the fact that the total amount of nitrogen fixed was often not measured resulted in a definitely misleading view as to the actual relative amounts formed in relation to the total amount of nitrogen fixed. An examination of all of the published experimental data shows that the yields of  $\text{NH}_3\text{-N}$  obtained actually never amounted to more than a fifth of the reported total nitrogen fixed and were ordinarily one tenth to one twentieth. The significance of this observation, previously overlooked, cannot be overestimated: the quantities of  $\text{NH}_3$  obtained were really so small, relatively, as easily to allow of their having been derived *entirely* from fixed

TABLE 14

*Experiments of Kostytshev, Ryskaltshouk, and Shvetsova (19) on the formation of  $\text{NH}_3\text{-N}$  and amino-N in Azotobacter cultures grown in free nitrogen*

CULTURE NO.	AGE	FINAL NET NITROGEN PER 100 CC.				PER CENT SOLUBLE-N OF TOTAL-N	PER CENT $\text{NH}_3\text{-N}$ OF TOTAL-N
		Total	Soluble	$\text{NH}_3\text{-N}$	Amino-N		
	days	mgm.	mgm.	mgm.	mgm.		
8	10	....	3.9	1.6	2.2	....	...
9	10	....	2.2	1.4	...	....	...
10	6	....	3.4	3.1	...	....	...
11	15	16.7	2.7	1.3	1.2	16.2	7.8
12	7	15.7	2.6	0.7	2.0	16.6	4.5
13	9	17.7	2.1	0.3	1.8	19.0	1.7
14	3	6.8	1.1	0.3	0.8	16.2	4.5
15	6	10.5	1.3	0.3	0.9	12.4	2.9
16	12	14.1	1.3	0.2	0.8	9.2	1.5
Average....	8.6	13.6	2.3	1.0	1.4	15.0	3.8

A. "*agile*" isolated from Petersburg garden soil. Pellicle normally formed in the 100 cc. 2 to 2.4 per cent mannite or glucose medium used per vessel and giving a layer 2 to 3 mm. deep. Analyses after centrifuging and filtering.

nitrogen compounds occurring as normal cell constituents of *Azotobacter*, by normal metabolic (chiefly catabolic) processes bearing no close connection to the mechanism of nitrogen fixation.

It has been possible to collect, in tables 14 and 15, *all* of Kostytshev's pertinent experiments. It will be seen that, in the first experiments which attracted so much attention (table 14), no more than 8 per cent  $\text{NH}_3\text{-N}$  of total-N was obtained, the yields of amino-N being, in fact, usually greater than  $\text{NH}_3\text{-N}$  by a factor of two- to threefold, and the total soluble-N being never more than 20 per cent of the total-N. Winogradsky did not report values for total-N, but it would appear from probable values based on organic substrate supplied that in general he obtained about the same yields of nitrogen fixed but somewhat smaller yields of  $\text{NH}_3$  than did Kostytshev. In 1930 (33) Winogradsky mentioned obtaining 3 to 4 mgm.  $\text{NH}_3\text{-N}$  per 2 to 2.5 gm. Na-lactate, which, with an estimated total fixation of 15 to 20 mgm. N (table 15), would correspond to 20 per cent  $\text{NH}_3\text{-N}$  of total-N; and in 1932 (34) there were reported: Experiments V, VI, VIII, and IV, 3.36, 1.68, 0.38, and 3.05 mgm.  $\text{NH}_3\text{-N}$  per 2.5, 2.5, 2 gm. Na-succinate and 10 cc. alcohol, after 6, 5, 7, and 7 days' growth.

TABLE 15

*Experiments of Kostytshev and Scheloumova (21) on the formation of  $\text{NH}_3\text{-N}$  in cultures of *Azotobacter* grown in free nitrogen*

CULTURE NO.	AGE	NET N IN CULTURE (MGM./GM. MANNITE = MGM./75 CC. GEL)			pH
		Total	$\text{NH}_3\text{-H}$	Per cent $\text{NH}_3\text{-N}$ of Total-N (calc. by B. & H.)	Final
I	days				
	2	6.93	0.07	1.0	7.1
	4	10.10	0.27	2.7	7.2
	6†	10.62	0.56	5.3	7.4
	12	9.24*	1.21	13.1 (24)†	7.7
	14	9.10*	1.23	13.6 (26)†	7.7
II	6	12.51	0.99	8.0	Not given
	8	12.44	0.78	6.2	Not given
	10	11.57	1.28	11.1	Not given
	12	12.97	1.66	13.1	Not given
III	4	6.93	0.14	2.0	Not given
	6	8.89	0.21	2.3	Not given
	8	10.01	0.42	4.4	Not given
	10	12.00	0.32	3.8	Not given
	12	12.93	1.47	11.4	Not given
IV	2	7.77	0.07	0.9	Not given
	4	9.04	0.14	1.5	Not given
	6	10.15	1.05	10.4	Not given
	8	11.83	1.36	11.6	Not given
V	4	9.85	0.63	6.4	Not given
	8	12.51	1.61	12.9	Not given
VI	6	9.65	0.98	1.0	Not given
	8	10.40	1.50	14.4	Not given
	12	10.66	2.49	23.3	Not given

*A. vinelandii* isolated from Krym soil. Grown in washed air on 150 cc. nitrogen-free silica gel blocks containing 2 gm. mannite, at 25°C. (III, 20°C.).

\* Loss of  $\text{NH}_3\text{-N}$  by volatilization in this single experiment (in others  $\text{NH}_3$  collected, or not lost).

† Calculated, by present writers, assuming total N = 10.62, and true  $\text{NH}_3\text{-N}$  value equal to observed value plus difference between 10.62 and observed total-N value.

‡ Kostytshev and Scheloumova remark, "The mannite was not consumed by the 6th day, and the ammonia formed during this time is therefore the first isolable product of nitrogen fixation [trans.]." It is very probable, however, that the mannite had by this time attained a very low figure. No value was measured, or cited, here or elsewhere in the paper, regarding the concentration of added organic substrate existing subsequent to addition. By general inference, some substrate remained in the blocks until at least the second to fourth day, or until the total nitrogen no longer increased, but, of course, not necessarily in all local areas of the gel. . . . Addition of sugar to cultures 4 to 6 days old resulted, by the eighth to tenth day, in 50 to 100 per cent more fixation of nitrogen, and a 60 to 80 per cent decrease in (but not total elimination of)  $\text{NH}_3\text{-N}$ .

In the great majority of the experiments, however, still smaller amounts were indicated, usually 0.01 to 0.05 mgm. of  $\text{NH}_3\text{-N}$  per culture (2 gm. substrate) per day, with a total liberation of 0.1 to 0.5 mgm. after a period of several days.

By ascertaining and then employing more optimum conditions, it was possible in the present experiments to obtain yields better than those just cited by a factor of two- to tenfold. Since it was possible to show clearly that the  $\text{NH}_3\text{-N}$  obtained even in this greater yield was not due in the slightest to a direct synthesis from  $\text{N}_2$ , it is concluded that all the  $\text{NH}_3\text{-N}$  obtained by the previous workers cited was likewise derived from cell-N. This conclusion would appear to deserve great weight since it was possible in the present investigation to repeat and confirm the findings of these previous workers when employing their technique. It cannot be urged that the results presented here are restricted in validity to the centrifuged suspensions employed in good part.

Winogradsky had advanced three reasons, all of them physiological or morphological, not chemical, for believing that the  $\text{NH}_3$  he obtained was ordinarily derived by direct synthesis from  $\text{N}_2$ : the cultures he observed were (a) young (3 to 7 days), (b) growing, (c) undegraded in appearance. As a matter of fact, however, by the time  $\text{NH}_3$ -formation had commenced, the cultures were long past the logarithmic phase of maximum growth rate characteristic of truly physiologically young cultures, and many or most of the cells were relatively quiescent. Increase in cell mass had in fact come practically to a standstill before  $\text{NH}_3$ -formation commenced, as a result of lack of organic substrate, a factor to which much less significance was attached than was merited. In regard to point c, we have already indicated that cells are little altered in outward appearance even after a demonstrated loss of 50 per cent of their nitrogen as  $\text{NH}_3\text{-N}$ , under conditions precluding any possible utilization of  $\text{N}_2$  during the process of loss ( $\text{H}_2$  replacing  $\text{N}_2$  in the gas phase). The formation of  $\text{NH}_3\text{-N}$  by macerated and toluene-treated cells, observed by Winogradsky, is likewise readily understandable, upon a basis of results presented in this paper, as simple decomposition, aerobic or anaerobic, of cell fixed nitrogen. Our observations in regard to pasteurization and the influence of toluene show that the enzyme systems concerned in deaminative decomposition are relatively insensitive to agents which have such marked inhibiting influences on more strictly vital processes such as growth. The relatively small yields obtained by Winogradsky eliminate any necessity, from the quantitative point of view, for invoking the postulate of direct synthesis from  $\text{N}_2$ .

Kostytshev had suggested that any  $\text{NH}_3\text{-N}$  obtained before practically complete consumption of substrate, was derived by direct synthesis from  $\text{N}_2$ . It is evident from table 15 that even under this condition he had never obtained more than 5, or even 3, per cent of the total N as  $\text{NH}_3\text{-N}$ . We have shown, however, that small amounts of  $\text{NH}_3$  may be obtained when the concentration becomes reduced to a certain low value; and in the heterogeneous medium employed by Kostytshev, substrate will fall to this value (or zero) in some local regions somewhat sooner than in others, so that Kostytshev's differentiation between primary and secondary  $\text{NH}_3$ -formation is without critical support. Furthermore, Kostytshev's belief that the presence of substrate completely prohibited deamination of added nitrogenous compounds, based on experiments with peptone and glycine only, will be shown elsewhere (14) to be entirely untenable also. Certain somewhat readily oxidizable nitrogenous compounds (e.g., arginine, asparagine, glutamate) compete successfully to some extent with added non-nitrogenous substrate and may undergo appreciable oxidation and  $\text{NH}_3$ -formation, depending upon certain very favorable conditions, chiefly of relative concentrations of nitrogenous and non-nitrogenous oxidizable substrate; and  $\text{NH}_3$ -formation from urea, which requires no oxidation, is entirely unaffected by oxidizable substrate. Such compounds might well occur sometimes in *Azotobacter*, in small (but only small) amounts, and also account for some of the small yields obtained in the presence of mannite by Kostytshev, in addition to the explanation of low (variable) concentration of mannite just offered.

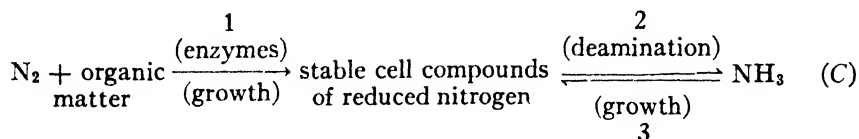
The same foregoing quantitative considerations would also appear to eliminate the 2-phase balance proposed in scheme A. It is exceedingly difficult to conceive of an equalization in the rates of the two steps, so maintained that, under the great variety of conditions to which

*Azotobacter* has been subjected, merely 20 per cent or less of the nitrogen involved should appear as  $\text{NH}_3$ . This becomes particularly evident when it is realized that the rate of growth in step 2 of scheme A can be varied ten to one hundredfold by a great variety of means [oxygen pressure, temperature, pH, concentration of organic and inorganic substrates, etc. (5)], means which can scarcely be held to affect step 1 independently of step 2, but nevertheless in just the same quantitative manner. If step 1 were a simple enzymic process, as pictured, there would appear to be no reason why, *sometimes at least*, this step should not proceed at a relative rate greatly in excess of that of step 2, with the formation of 5, 10, or 100 times as much  $\text{NH}_3$ -N as cell-N, the cells acting simply as catalytic agents. The theory of 2-step balance is clearly untenable, even pictorially.

Similarly, no quantitative experiments were advanced to demonstrate the operation of the mass law in a reversible synthesis of  $\text{NH}_3$  from  $\text{N}_2$ , as suggested in scheme B. The incorrectness of this proposition is evident from the fact that addition of  $\text{NH}_3$  to growing *Azotobacter* cultures, in any concentration greater than that necessary to prevent  $\text{N}_2$ -assimilation, has never resulted in any observed liberation of  $\text{N}_2$  (5). The well-known inhibiting action, on fixation, of added fixed nitrogen compounds such as ammonia, nitrate, and peptone, cited by Kostytshev, or others (adenine, asparagine, urea, glutamate, alanine, etc.), is readily interpretable upon a basis of preferential substrate utilization; that is, these fixed nitrogen compounds are simply more readily available. This partly kinetic, rather than purely thermodynamic, phenomenon is of widespread physiological occurrence with respect to all types of substrates, nitrogenous or otherwise. The fact that such a great variety of fixed nitrogen compounds inhibit fixation just as ammonia does, would appear to render any specific operation of the mass law in regard to  $\text{NH}_3$  uncalled for. Finally, in our suspensions undergoing deamination, in the absence of added organic substrate, we have never observed any loss of total fixed (Kjeldahl) nitrogen in enclosed suspensions in which large amounts of  $\text{NH}_3$  have accumulated; nor has accumulated  $\text{NH}_3$ , or added  $\text{NH}_3$ , had any measurable inhibiting action on the further formation of  $\text{NH}_3$ , up to at least 2000 mgm./l.

#### *Proposed mechanism of $\text{NH}_3$ -formation*

The following mechanism is herewith proposed as consistent with all the findings presented in this paper and with all those obtained by previous investigators:



$\text{NH}_3$ , as such, is by this scheme very definitely not the first product of fixation which it might be possible to isolate. Growth, and elaboration of cell nitrogen compounds such as protein, amino acids, nucleic acid, amides, etc., occur to some (undetermined) extent by step 1, without the necessarily prior intervention of  $\text{NH}_3$ ; and growth may also occur with  $\text{NH}_3$  definitely derived in step 2 from compounds produced in step 1. Any  $\text{NH}_3$  produced in step 2 and not utilized by step 3 in growth would appear as extracellular ammonia and account for that heretofore observed.

Scheme C does not attempt here to distinguish in step 1 between stages in growth proper, and stages in the chemical mechanism of fixation proper [involving azotase and nitrogenase (5)]. But it clearly places the formation



of any *previously observed* extracellular  $\text{NH}_3$  *subsequent* to the formation, from  $\text{N}_2$ , of some other stable cell compound, or compounds, of reduced fixed nitrogen. The possibility of  $\text{NH}_3$  being formed in Step 1 (with or without growth occurring) cannot, of course, be eliminated, but as already extensively indicated, no critical data in support of this are *at present* available, although the work of Bach, Yermolieva, and Stepanian (2), in which large yields of ammonia were reported with "non-living," cell-free preparations of *Azotobacter*, is suggestive in this connection.

TABLE 16

*Formation of non- $\text{NH}_3\text{-N}$  during the course of ammonification of cell suspensions (Burk and Horner)*

TIME	DRY MATTER PER CC.	N*	TOTAL-N PER CC.	$\text{NH}_3\text{-N}$ IN FILTRATE PER CC.		SOLUBLE N IN FILTRATE PER CC.	NON- $\text{NH}_3\text{-N}$ PER CC.	PER CENT TOTAL N AS	
				Ness.	Dist.			$\text{NH}_3\text{-N}$	Non- $\text{NH}_3\text{-N}$
hours	mgm.	per cent	mgm.	mgm.	mgm.	mgm.	mgm.		
22	....	....	0.350	.....	0.062	0.121	0.059	18.0	16.8
46	....	....	0.340	0.085	0.100	0.175	0.075	27.2	21.9
70	1.13	15.6	0.340	0.110	0.124	0.164	0.040	34.4	11.7
91	....	....	0.318	0.135	0.143	0.167	0.024	43.8	7.6
140	....	....	0.314	0.145	0.134	0.164	0.030	44.4	9.6
15 days	0.89	13.1	0.332	0.187	0.189	0.215	0.026	56.5	7.8
25 days	0.65	17.3	0.319	0.187	0.205	0.208	0.003	61.2	0.9

\* Based on total N minus total soluble N in filtrate.

4-day *A. vinelandii* culture made up into 150 cc. stock suspension containing initially 0.340 mgm. cell-N per cc., and 2.26 mgm. cell dry matter (D.M.) per cc. (15 per cent N). Incubated in organic matter-free medium at pH 7.8 and 32°C., in a closed Erlenmeyer flask. Gas phase, air. Samples withdrawn (at varying times) for analysis for total N (Kjeldahl), and then filtered through celite and analyzed for  $\text{NH}_3\text{-N}$  (Nessler's and microdistillation), total soluble-N, and non- $\text{NH}_3\text{-N}$  (by difference).

Notes: The dry matter decreased to less than one third, whereas the total N and nitrogen percentage in the dry matter remained essentially constant, error being considered. The Nessler and microdistillation methods for determining  $\text{NH}_3\text{-N}$  gave essentially agreeing results. Initially the soluble, non- $\text{NH}_3\text{-N}$  is almost of the same order as the  $\text{NH}_3\text{-N}$ , but eventually it all disappears, practically.

It might be thought that, *a priori*,  $\text{NH}_3$  is *necessarily* involved to a very large extent in growth and protein formation. However, many fixed nitrogen compounds provide a faster rate of growth than  $\text{NH}_3$ , and hence it could appear that they are utilized, for the most part at least, without the nitrogen in them being first converted to  $\text{NH}_3\text{-N}$ . By the same line of reasoning, although  $\text{N}_2$  is essentially completely reduced, it is not *strictly* necessary for  $\text{NH}_3$  itself actually to be one such reduced compound involved. We have indicated elsewhere that some amide may possibly be specifically involved (7, 8). It would appear desirable to take the view that in fixation several successive reactions are involved, taking place in very dilute solution, the various intermediate compounds formed normally having a very brief existence and being rapidly changed into some further product; consequently, whereas the initial substances involved and to some extent the final products of the reaction may be

known, there will be comparatively little known as to the various stages in the formation of the final product, and that information will be determined probably by rather special means which will in any event require the closest analysis and circumspection.

Step 2 undoubtedly takes place, in part, through the intermediate formation of extracellularly liberated, fixed nitrogen compounds, such as have been observed by Kostytshev (table 14), Halversen (12), and Ranganathan and Norris (29). We have observed their accumulation to the extent of 10 to 75 per cent of the  $\text{NH}_3\text{-N}$  (table 16). Their actual concentration apparently increases at first, as a function of time, and then decreases, as their conversion to  $\text{NH}_3\text{-N}$  becomes nearly complete (90 per cent). Kostytshev had believed on the basis of the results given in table 14 that extracellular amino-N was formed in a synthetic process from the  $\text{NH}_3\text{-N}$  (itself derived directly from  $\text{N}_2$ ), but from results of this paper it is very probable that the converse is the case, namely, the extracellular amino-N is formed in a degradation process and then by oxidation and hydrolysis further gives rise to some or most of the  $\text{NH}_3\text{-N}$  observed. This matter is being studied further.

*Postscript added to galley proof.* Shortly after submission of this paper to press there appeared an article by Winogradsky, "The Method in Soil Microbiology as Illustrated by Studies on Azotobacter and the Nitrifying Organisms" (*Soil Sci.* 40: 59-76, 1935). In this article Winogradsky gives an excellent characterization of the differences in outlook and method necessarily involved in studying physiological, chemical, industrial, or medical bacteriology on the one hand and soil microbiology (agrobiology) on the other. It is our opinion that the agrobiological method, so advantageous in studying the activity of Azotobacter in its natural environment, can be of but limited service in attacking the essentially biochemical problem under immediate concern here, namely, the significance of ammonia formation in its relation to the mechanism of nitrogen fixation. As indicated in detail throughout this paper, it is difficult if not impossible in the agrobiological method to carry out a number of different types of control experiments necessary in interpreting observations bearing on the " $\text{NH}_3$  theory" of Kostytshev and Winogradsky. Winogradsky finds the " $\text{NH}_3$  theory" opposed to the "Azotase theory" (5), but this is difficult to understand, since the former is concerned with intermediate products possibly involved in fixation and the latter with enzymic catalysts possibly involved. He states that the "Azotase theory" is based "on the non-production of soluble nitrogen as an essential condition," but no claim has been made as to any such necessary condition, only that it was a normal one, and in the original description of azotase (and nitrogenase) it was stated that "freedom from growth limitation, and extracellular isolation, are eventual aims" (5, p. 24). The implications of the since published work of Bach, Yermolieva, and Stepanian (2) lie in this direction, and opportunity may be taken here to state, briefly, as a result of recent direct contacts established through the very great courtesy of Academician Bach and his co-workers, that the outlook is very promising.

#### SUMMARY

In this paper are presented the results of a detailed and widely varied investigation concerning the chief qualitative and quantitative factors governing the extracellular production of ammonia by Azotobacter grown in free and various forms of fixed nitrogen. Both *A. vinelandii* and *A. chroococcum* have been employed, with no essential differences observed. It is shown that the extra-

cellular ammonia *observed so far, in our own and in previous investigations*, is derived in all probability entirely from decomposition of normal cell nitrogen and not, in any measurable quantity, by direct synthesis from free  $N_2$ . In this connection a quantitative critique has been given of all previous theories bearing on the origin of the ammonia, and the major previous experiments have been repeated and confirmed. As a result of *much additional experimentation*, it is shown that the view, previously advanced, that ammonia occurs in reversible (mass law) equilibrium with  $N_2$  is untenable; and that likewise the view that the ammonia is produced from a disturbed balance between primary synthesis from  $N_2$  and utilization in growth is also untenable. The ammonia observed has been liberated *after*, not *before*, a synthesis into cell nitrogen. The occurrence of any ammonia as an essential intermediate product in the fixation of nitrogen, *although possible, still remains to be demonstrated*. In support of these several conclusions, the following specific findings have been obtained:

Under optimum conditions *Azotobacter* liberates aerobically a maximum of 50 per cent of its cell nitrogen as ammonia, and anaerobically a maximum of 10 per cent. The optimum pH is 7.8 to 8.0, with a very broad, effective range exceeding the limits of pH 5 and 10. The optimum temperature is 30° to 40°C., with a very broad range exceeding 10° and 50°. The presence of  $N_2$  gas for extensive ammonia formation is totally unnecessary once the cell nitrogen has been formed.  $H_2$  is likewise inert. The influence of  $O_2$  in oxidation of cell material is independent of the pressure between 0.01 to 1 atmosphere.

By far the most important factor for optimum ammonia formation is elimination of oxidizable substrate from the medium, either slowly by respiration, or quickly by mechanical separation (centrifugation). Most oxidizable organic matter inhibits the formation of ammonia completely at the low concentration of 0.03 per cent or less. Upon quick mechanical removal of substrate from the growth medium of cultures of whatever *appearance or age* (1-30 days), ammonia formation sets in immediately and, under optimum conditions, without lag in rate. The time course follows a first order (logarithmic) course, with a specific rate of decomposition of 2 per cent of the transformable cell nitrogen per hour (1 per cent of the cell nitrogen), with only about a day required to obtain half of the final, maximum possible decomposition.

The process of aerobic ammonia formation is at all times closely related to the *oxidation of cell material*, corresponding approximately to protein oxidation, since with ammonification carried to completion, the respiratory quotient ( $CO_2$  produced/ $O_2$  consumed) is 0.8 to 0.9, and 4.5 mols of  $O_2$  are consumed per mol of  $NH_3$  formed. Ammonia formation and oxidation of cell material in the absence of substrate follow the same general time courses and show a remarkable degree of correlation in relation to inhibition by a large number of agents, including *toluene*, cyanide, very high nitrate concentration, very low oxygen pressure (below 0.01 atmosphere), pasteurization, adverse pH, and adverse temperature. Inhibition by added organic matter (sugars, organic acids) may be divided into three classes: substrates themselves oxidized and inhibiting both ammonia formation and oxidation of cell material; substrates not oxidized but nevertheless inhibiting these processes; and substrates not oxidized and not inhibiting. The simultaneous inhibition of both ammonia formation and cell oxidation ordinarily occurs, for any given organic substrate, over the *same* concentration range, which, for the first substrate class, is usually 0.01 to 0.03 per cent and, for the second substrate class, 1 to 10 per cent.

The overall process of anaerobic formation of ammonia is hydrolytic, and carbon dioxide is normally not formed. Anaerobically there is no inhibition by toluene or (so far as tested)

by any of the three classes of organic substrates mentioned in connection with aerobic inhibition.

Cultures grown previously in fixed nitrogen, such as nitrate, ammonia, peptone, urea, asparagine, glutamate, adenine, creatine, or alanine, and then transferred to an environment containing *neither fixed nor free nitrogen* (the latter replaced by hydrogen gas), yield ammonia quantitatively in essentially the same manner, both aerobically and anaerobically, as cultures grown with  $N_2$ . This broad finding, in particular, shows that the mere occurrence of ammonia in cultures of *Azotobacter* grown in  $N_2$  cannot be regarded as critical evidence in favor of a view current that the ammonia observed is derived, either wholly or in any part, specifically and directly from  $N_2$ .

From the agronomic point of view this paper demonstrates that a large part of the nitrogen which might be fixed by *Azotobacter* in soils would be liberated in a form readily available to plants, spontaneously, without the necessary intervention of other microbiological agents. By virtue of its heterogeneity the soil will provide at one time or another conditions satisfactory for the spontaneous liberation from *Azotobacter* cells of ammonia, and, to some extent, other soluble nitrogenous compounds. It is likewise shown that *Azotobacter* cells are able to ammonify vigorously, and generally with oxidation, a large number of fixed nitrogen compounds, including many amino acids and proteins.

It is probable that the majority of findings obtained in this investigation with respect to *Azotobacter* would be found to be characteristic of many soil organisms, if a similar detailed study were made. The findings are in every instance consistent with known chemical, physiological, and bacteriological phenomena and should be readily repeatable.

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# A PRACTICAL ANTIMONY ELECTRODE FOR SOIL pH DETERMINATION

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The antimony electrode is attractive to the soil investigator, since it offers a means for the potentiometric determination of pH without the addition of any active chemical or gas, and with equipment which may be simple and very rugged in construction, in contrast to the delicate apparatus required for the glass electrode method. Although the antimony electrode has not the unique advantage of the latter, viz., absolute immunity to disturbance from redox potentials which affect all metal electrodes, the oxidizing substances of normal soils are so insoluble that they appear to have no effect upon the antimony, however active they may be toward quinhydrone in solution.

A comparative study of methods for the determination of soil pH (4), in which data obtained by the use of the glass electrode were taken as the standard, has indicated that the antimony electrode is quite satisfactory, much superior in accuracy to the quinhydrone method and modifications thereof, as well as various colorimetric methods applied to soils, but apparently significantly inferior to the hydrogen electrode in point of accuracy. The mean of all determinations on 17 samples was pH 5.10 for the glass, 5.07 for the hydrogen, 5.24 for the quinhydrone, and 5.01 for the antimony electrode. These figures are consistent with the expected trend of errors of the several methods—the soft glass membrane may supply soluble alkali, streaming  $H_2$  removes  $CO_2$ , quinhydrone reduces  $MnO_2$  to  $Mn(OH)_2$ , and the technic of the antimony electrode method ensured equilibrium with the laboratory air with respect to  $CO_2$  content.

No review of the already considerable literature on the antimony electrode in soil work will be attempted, as this has been sufficiently covered in previous contributions from this laboratory (1, 4). The theory of the antimony electrode is discussed by Roberts and Fenwick (6), who showed that under ideal conditions, including exclusion of air, the relation of potential to pH is as predicted, between pH 2 and 10, with 24 hours allowed for equilibrium. The latter requirement as well as the technic in general employed by these authors is obviously impractical for rapid measurements on soil suspensions. In application to the latter, Hooghoudt (3) compared various forms of electrodes, including the electrodeposited metal recommended by Roberts and Fenwick, and concluded that in a solution exposed to air a simple cast rod is as good as

any, and that a special addition of antimony trioxide is unnecessary. Nearly all who have worked upon soils have used the cast rod electrode, mostly without addition of oxide. In reviewing the published accounts of the behavior of the antimony electrode in buffers and soil suspensions, etc., one is struck by the fact that all recommend it for practical use and give equations for calculating indicated potentials to pH values, without agreement in the latter. The fact that the potential-pH relation of the cast antimony electrode is not a constant is generally recognized by those who have studied its practical application; the equations specified are applicable only under narrowly prescribed conditions. Snyder (7, 8), for example, specified that the potential be read while the suspension is being shaken; Hooghoudt (3) considered rotation of the electrode preferable; Barnes and Simon (1) recommend that the soil suspension be allowed to settle, and the electrode be thrust to the bottom with a reading 10 seconds later, checked by rotating the container a few times and reading after 10 seconds, repeating to constancy. The writer's experience has been that the most consistently reproducible potential differences were obtained with a suspension so heavy that it did not settle quickly, thoroughly but not violently stirred about the electrode, then allowed to rest with the electrode near the bottom for one minute before being read.

The potential assumed by an antimony electrode in a buffer or soil suspension appears to depend to a considerable extent upon the state of the electrode surface, as well as upon the pH of the medium. If the metal is tarnished or covered by a film of oxide, the potential may be very different from that shown by a highly polished electrode. Vlès and Vellinger (10) observed that a newly polished electrode placed immediately in a buffer exposed to air has a reproducible potential, which changes with time in a regular manner, whether or not the electrode is in the buffer; they conclude that the crystals of metal at the surface are placed in a state of strain by the polishing operation, from which they gradually recover with accompanying change in electrical characteristics. Vlès (9) sought to take advantage of this constant rate of change by using two electrodes with identical treatment, the one in a buffer of known pH as the standard electrode. Hooghoudt (3) reported discouraging results with this arrangement in soil work, and trials in this laboratory were no more successful. It seems probable that the factor of mechanical abrasion by soil particles may be important. The writer has noted that an electrode kept in a 1:2 suspension of a silt loam soil mechanically stirred at a rate just sufficient to prevent settling soon comes to a state of comparatively slow change in potential; if now the electrode be rotated half around, so that the side formerly protected is exposed to abrasion, the potential may change by 10 mv. or more, with slow drift as before. This effect was not noted in clear buffer solution. Rapid changes from soil suspension to buffer, both stirred in the manner described, were productive of consistent differences in potential, indicating that the change was more in the electrode than otherwise, although Hooghoudt (3) considers increase in antimony content of the solution an impor-

tant cause of drifting potential. It is difficult to reconcile this opinion with the repeated observation that an addition of antimony trioxide to the solution has no consistent effect upon electrode potentials. However, it is generally agreed that the electrode is unreliable in the presence of acids at pH values below 2 and in the presence of substances, e.g., hydroxyacids, having a specific solvent power for antimony. Citric acid, a constituent of some buffer mixtures, is of this class.

The observation that *differences* in potential shown by the rod antimony electrode in suitable buffers taken as standards and other buffers or soil suspensions were consistently near the expected values when the measurements were made in rapid sequence, suggested that it might be practical to employ the antimony electrode in a manner analogous to the accepted technic with the glass electrode, i.e., with frank recognition of the fact that the electrode has no fixed characteristic but is constantly likely to change. But whereas the characteristics of a glass film suitable for use as an "electrode" closely approximate constancy, or at least change very slowly because of inherent chemical stability, this cannot be claimed for the antimony electrode. Nevertheless, with a suitable uniform and rapid technic, experience has indicated that the change in the antimony electrode is sufficiently regular that indications of satisfactory accuracy for most soil work can be depended upon. When operating in this manner, a crucial point is whether or not the electrode is sufficiently sensitive to *change* in pH, i.e., is not sluggish or subject to lag. Best (2) has stated that this is a serious defect of both the antimony and quinhydrone electrodes, and several other authors have stated that an antimony electrode heavily tarnished is slow in response to changes. Using a reasonably clean electrode, the writer has noted no difficulty from this source. In work with soils, the slight abrasion by mineral particles seems to be sufficient to keep the surface in good condition, although after long disuse or contact with a solution causing a heavy tarnish to form, a cleaning with No. 00 sandpaper may be advisable. A newly scoured electrode is subject to rapid change, however, and the indications are likely to be less reliable than those obtained after some use.

With the foregoing in mind, a simple assembly of apparatus has been designed, enabling the use of a rod electrode in soil suspensions and buffers in rapid sequence, with agitation as desired and maximum protection of the electrode against external contacts and changes in environment. An outer mantle is made by drawing out and cutting off the closed end of a Pyrex test tube and sealing on a side arm for attachment of a rubber pipet bulb, as sketched in figure 1. By means of a perforated cork, the antimony electrode mounted in a straight glass tube is supported within the mantle, as shown. A suitable electrode is easily made by drawing the end of a 6-7 mm. i. d. Pyrex tube to a short rounded cone and cutting off to about 10 cm. length. Fragments of C. P. antimony are dropped into the open end and melted over a burner until the casting is about 3 cm. long. The tube is then set in a crucible filled with



sand in a furnace at 650–700°C., somewhat above the melting point of antimony, and allowed to cool slowly. By cautiously reheating over a burner and cooling in water, the glass may be cracked off with little risk of breaking the very brittle casting. Electrodes made thus are coarsely crystalline and, in the writer's experience, superior in performance to these suddenly cooled from the

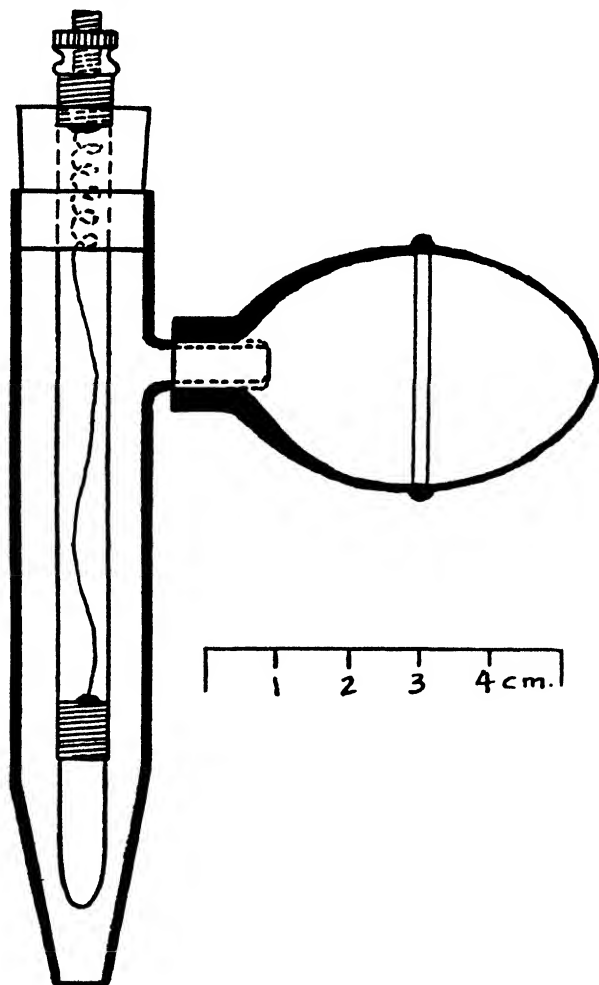


FIG. 1. ANTIMONY ELECTRODE

molten state; the latter are finely crystalline and are much less likely to break. A copper wire is carefully soldered to the antimony with ordinary solder, rosin being used for flux, and about 1 cm. of that end sanded until it will readily enter a length of the same tubing. The metal and tube are then warmed and coated with sealing wax and fitted together. A screw binding post from a discarded dry cell may be cast into a plug of solder, to which the other end of the wire is

attached, and fastened into the top of the tube with wax, for a neat external connection. The exposed surface of the antimony is sanded smooth with No. 00 sandpaper before the parts are completely assembled.

The antimony electrode is invariably connected to the negative binding post of the potentiometer. A saturated potassium chloride electrode with KCl-agar filled siphon or ground glass-plugged tube filled with crystals and solution for a salt bridge completes the cell. The sample of soil with sufficient water to make a mud easily drawn up into the mantle is contained in a 50-ml. beaker. With air-dry soils, it is well to prepare this mixture a half hour or so beforehand, as a tendency for pH to change rapidly at first is often noted. The suspension is thoroughly stirred with a rod, then drawn up into the mantle some distance above the exposed antimony, expelled and drawn up again a few times. The filled electrode vessel is finally allowed to stand with its tip in the mud remaining in the beaker. The siphon tube is then brought into contact with the latter, and the potential read one minute after the suspension was allowed to come to rest. The electrode is washed quickly by drawing up and expelling distilled water a few times, and the potential in a phosphate buffer is determined in the same way. The pH of the soil suspension is calculated by use of the formula

$$\text{pH}_X = \text{pH}_B - \frac{E_B - E_X}{0.000198322 T}$$

in which the subscripts *B* and *X* refer to buffer standard and unknown respectively, *E* is the indicated potential in volts, and *T* is the absolute temperature,  $273 + t^\circ\text{C}$ .

The factor 0.000198322 *T* is common to all electrode equations, indicating the effect of temperature upon the relation between differentials in potential and pH, or  $dE/d\text{pH}$ . As numerous investigators have noted that this does not always have the theoretical or even a constant value over the entire pH range for the cast rod antimony electrode, it will probably be more accurate to standardize the electrode in *two* buffers, as closely as possible bracketing the pH of the sample. Then the observed value of  $dE/d\text{pH}$  may be substituted;

$$dE/d\text{pH} = \frac{E_{B_1} - E_{B_2}}{\text{pH}_{B_1} - \text{pH}_{B_2}}, \text{ and } \text{pH}_X = \text{pH}_B - \frac{E_B - E_X}{dE/d\text{pH}}$$

In order to avoid confusion in calculation, the buffer with numerically greater pH value is designated *B*<sub>1</sub>; the other, *B*<sub>2</sub>; and the soil sample, *X*; the corresponding potentials (*E*) and pH values are designated by these subscripts. Potentials expressed in decimal volt and pH values are considered positive, but the calculation is algebraic with regard to sign. This procedure has the advantage that all necessity for an uncertain temperature correction is avoided, provided there is constancy during the three measurements with the antimony electrode.

Phosphoric acid-sodium phosphate buffers suitable for standards may be prepared by mixing 0.2 *M* H<sub>3</sub>PO<sub>4</sub> and 0.2 *N* NaOH and diluting. A 1.0 *M*

stock solution of phosphoric acid is first prepared by diluting 68.5 ml. of the C. P. reagent (85 per cent) to 1 liter, and a portion is further diluted to 0.2 *M*. An aliquot of the latter is titrated with 0.2 *N* NaOH to a clear yellow with methyl orange; the same volume will be required if the dilution is correct, and, if not, the proper dilution is calculated. To 250-ml. portions of the exactly 0.2 *M* phosphoric acid are added quantities of 0.2 *N* NaOH as in table 1, and the mixture is diluted to 1 liter. The exact pH value of each mixture is determined, conveniently by the quinhydrone method,<sup>1</sup> and employed in the calculation of the pH of the sample, as outlined.

TABLE 1  
*Phosphate buffer solutions*

pH	0.2 <i>N</i> NaOH	pH	0.2 <i>N</i> NaOH
	<i>ml.</i>		<i>ml.</i>
2.0	100	6.0	280
3.0	225	7.0	400
4.0	250	8.0	485
5.0	255	9.0	500

TABLE 2  
*Data for antimony electrodes in buffers and soil suspensions*

ELECTRODE NO.	POTENTIALS IN BUFFER SOLUTIONS					IN SOILS. <i>E<sub>X</sub></i>	INDICATED pH <sub>X</sub>
	<i>E<sub>B1</sub></i>	pH <sub>B1</sub>	<i>E<sub>B2</sub></i>	pH <sub>B2</sub>	<i>dE/dpH</i>		
	<i>volt</i>		<i>volt</i>			<i>volt</i>	
1 (a)*	0.294	5.19	0.202	3.67	0.061	0.242	4.33
2 (b)	0.300	5.19	0.209	3.67	0.060	0.247	4.31
3 (c)	0.301	5.19	0.213	3.67	0.058	0.250	4.31
(d)	0.303	5.19	0.214	3.67	0.059	0.251	4.30
(e)	0.307	5.35	0.218	3.86	0.060	0.248	4.36
(f)	0.305	5.35	0.219	3.86	0.058	0.249	4.38

\*(a) A rapidly cooled electrode, heavily tarnished when used.

(b) A similar electrode, clean.

(c) A slowly cooled electrode, clean.

(d) Same electrode, immediately after.

(e, f) Same electrode, several days later, different buffers.

The distilled water for preparing buffers and soil suspensions should be freed from CO<sub>2</sub> by aeration rather than by boiling, as the necessity of having as nearly as possible the same content of dissolved air in both has been emphasized by Parks and Beard (5). For this reason, a buffer solution in which molds have grown should be discarded or at least thoroughly aerated before use. The author has obtained some erratic indications from laboratory treated soils, which he is inclined to attribute to deficiency of dissolved oxygen in the sus-

<sup>1</sup> If the same calomel electrode and siphon tube are used, the latter should be well cleaned of traces of quinhydrone which may adhere, before being used again with the antimony electrode.

pensions, as the indicated pH values were much too high. The sensitivity of the antimony electrode to variations in oxygen content is a serious defect that may be demonstrated by the addition of a drop of dilute  $\text{H}_2\text{O}_2$  to a buffer, greatly altering the potential of the electrode therein and often reversing its polarity with respect to the calomel electrode. Unlike the quinhydrone electrode, however, it is not appreciably affected by the insoluble oxidants of normal soils.

In illustration of results to be expected under favorable conditions, data obtained from tests on a silt loam soil and phosphate buffers are presented in table 2. Determinations on the same soil by Dr. J. A. Naftel, made under comparable conditions, indicated pH 4.32 and 4.24 for the glass electrode, 4.34 and 4.31 for the hydrogen electrode, and the author obtained 4.48 and 4.47 in determinations with the quinhydrone electrode. Colorimetric determinations, which were more consistent with this soil than is generally the case, indicated pH 4.1–4.2.

#### SUMMARY

A form of cast rod antimony electrode in a syringe-like protective mantle and the technic of rapid pH measurements on soil suspensions are described. The essential principle is a practically simultaneous standardization in a suitable buffer of known pH, in recognition of the fact that when used in this manner the potential-pH relation of the antimony electrode is not absolutely constant, but sensitivity to changes in pH and constancy in characteristics over short periods, even with change from one solution to another, are sufficient for determinations of satisfactory accuracy.

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# A NEW METHOD FOR TESTING THE PURITY OF MARLS AND LIMESTONES<sup>1</sup>

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The method that is at present generally used for determining the purity of marls and limestones consists of treating a weighed amount of the materials with dilute acid and measuring by gaseometric methods the carbon dioxide evolved.

A method for determining the purity of marls and limestones, based upon an entirely different principle from the gaseometric method and having some distinct advantages over it, is here presented. This new method has for its principle the measurement of the specific gravity of calcium chloride solution formed when definite amounts of marl or limestone are treated with a definite quantity of hydrochloric acid of known strength. With a knowledge of the specific gravity of the acid and of the calcium chloride solutions formed when equal weights of pure calcite crystals and of marl or limestone are dissolved in equal quantities of acid, the purity of the marl or limestones can readily be calculated.

## PROCEDURE OF METHOD

The exact procedure of the method consists first of preparing a stock solution of hydrochloric acid by mixing distilled water and concentrated HCl having a specific gravity of 1.18 at the rate 650 cc. of water to 350 cc. of acid. To every liter of the stock acid solution is added 15 cc. of amyl alcohol in order to prevent frothing when samples of limestone or marl are dissolved in it. The specific gravity of this stock acid solution is then determined by a hydrometer devised specially for this method.

For purposes of standardization, exactly 16 gm. of calcite crystals are weighed into a beaker and treated with 50 cc. of the acid solution. After the reaction is completed, the liquid is poured into a 50-cc. cylinder and its specific gravity determined.

The purity of marl and limestone is now determined by following exactly the same procedure. The only difference is that in the case of the marl and limestone there may be sediment in the liquid; and in order that this may settle out, the liquid is allowed to stand for about three minutes before its specific gravity

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is determined. It was found that any sediment present tends to settle out very rapidly, and it is not necessary to filter the liquid except in special cases. Various trials showed that the difference in specific gravity between filtered and unfiltered liquids from marls and limestones did not amount to more than 0.5 per cent of  $\text{CaCO}_3$ .

The procedure followed for calculating the percentage of purity from the hydrometer readings is as follows: Supposing that the corrected hydrometer reading of the acid stock solution is  $10^\circ$  Baumé, that of the calcite-acid product is  $20^\circ$  Baumé, and that of the marl-acid product is  $15^\circ$  Baumé. The  $10^\circ$  Baumé is subtracted from each of the other two readings. Then the difference in degrees Baumé for the marl-acid product is divided by the difference in degrees Baumé of the calcite-acid product, and the result is multiplied by 100. The product is the percentage of purity of the marl.

In view of the various complications involved in the case of soils, this method is applied at present only to soils that are highly calcareous.

TABLE 1

*Comparison between hydrometer method and gaseometric method for determining purity of marls and limestones*

SAMPLES	GASEOMETRIC METHOD	HYDROMETER METHOD
	<i>per cent</i>	<i>per cent</i>
Marl 1.....	76.1	78.5
Marl 2.....	97.3	95.9
Marl 3.....	95.2	94.6
Marl 4 (dolomitic).....	93.8	93.2
Limestone.....	97.6	98.1
Calcareous Soil 1.....	42.5	40.2
Calcareous Soil 2 (dolomitic).....	30.5	30.2

For greater convenience and higher accuracy the reading of the hydrometer is taken at the surface of the liquid column.

The hydrometers that were specially devised for this method are small and are calibrated to read in  $0.1^\circ$  Baumé. They come in series, one reading from  $5^\circ$  to  $10^\circ$ , one from  $10^\circ$  to  $15^\circ$ , and one from  $15^\circ$  to  $20^\circ$  Baumé.

It so happens that solutions of  $\text{CaCl}_2$  and  $\text{MgCl}_2$  prepared from pure  $\text{CaCO}_3$  or  $\text{MgCO}_3$ , as previously described, have practically the same specific gravities, 1.157 for the former and 1.177 for the latter. This fact makes it possible to determine the purity of dolomitic marls and limestones with little error when calculations are based on the specific gravity of  $\text{CaCl}_2$  solutions.

As temperature exerts an influence on specific gravity, the temperature of the liquid is taken every time its specific gravity is measured. It was found that  $1^\circ\text{F.}$  makes a difference of about 0.04 graduation on the hydrometer. Since the hydrometer is calibrated at the temperature of  $60^\circ\text{F.}$ , all hydrometer readings are reduced to this temperature, and calculations are made on this basis.

For temperatures above 60°F. the corresponding corrections are added to the hydrometer readings and for temperatures below 60°F. they are subtracted.

This method is accurate to about 0.5 per cent of  $\text{CaCO}_3$ . By using the strength of acid and quantity of marl or limestone already mentioned, the sensitiveness of the method is increased so that 0.1 graduation on the hydrometer is equal to about 1 per cent  $\text{CaCO}_3$ , and the hydrometer can be easily read to 0.05.

In table 1 is shown a comparison between the hydrometer method and the gaseometric method for determining the purity of some typical marls and limestones.<sup>2</sup>

An examination of the data in table 1 reveals at once the fact that the hydrometer method gives approximately the same percentage of purity of the marls and limestones tested as does the gaseometric method. The small differences shown in some cases have no significance in view of the fact that in the gaseometric method only 0.25-gm. samples were used whereas in the hydrometer method 16-gm. samples were used. Hence, if these small differences have any significance they should be in favor of the hydrometer method.

The hydrometer method appears to have at least two distinct advantages over the gaseometric method: first, in the hydrometer method a 16-gm. sample is used, whereas in the gaseometric method only from 0.25 to 2.00-gm. samples are used; secondly, where there are a large number of samples of marls and limestones to be analyzed the hydrometer method is simpler and much quicker because a large number of samples can be run at the same time depending upon the number of beakers and cylinders available, whereas in the gaseometric method only one sample at a time can be run.

#### SUMMARY

A new method is presented for determining the purity of marls and limestones.

The principle of this method is based upon determining the specific gravity, by means of the hydrometer, of the calcium chloride solutions formed when marls and limestones are treated with HCl.

A knowledge of the specific gravity of the acid and of the calcium chloride solution formed, when pure calcite crystals are treated with the acid, permits ready calculation of the purity of the marls and limestones.

The method was compared with the gasometric method, and both gave approximately the same results.

<sup>2</sup> In this comparison the writer is greatly indebted to O. B. Winter, of the Chemistry Experiment Station, who performed the gaseometric determinations.





# SOIL SWELLING: I. THE SWELLING OF SOIL IN WATER CONSIDERED IN CONNECTION WITH THE PROBLEM OF SOIL STRUCTURE

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Absorption of water by soil, leading to an increase of volume, is in many cases accompanied by the very complex phenomenon of a change in soil structure. This acquires a special interest because it presents possibilities of utilizing the phenomenon of soil swelling for the elucidation of the structure problem from a new point of view and for finding methods allowing a numerical expression of the changes taking place during this swelling. The problem of the connection between the phenomenon of swelling and the structure of substance is not a new one for chemical technology and the chemistry of colloid substances.

Bütschli (2) has shown, on many examples of swollen substances studied under the microscope, that a change takes place in their structure. Interesting studies in this field have lately been carried through in which microscopic X-ray and cinematographic technique has been applied; by Hess, Trogus, et al. (7); by Katz and Mark (12) in the microscopic and roentgenoscopic study of swollen ramie fiber; by Hess and Rabinowitch (6) in a study on the swelling of different kinds of cotton fiber in which micro-cinematographic filming was applied.

Of particular interest for soil science are the researches of Hofmann, Endell, and Wilm (3) on the swelling of an argillaceous mineral montmorillonite. This mineral is the main component of bentonite clays, found often in soils. The authors have demonstrated that in the swollen montmorillonite all the interferences, except the one with a very small angle of reflection (001), remain in the same position, whereas the latter is displaced to an extent varying with the change in the water content. The same phenomenon of "crystal swelling" has been observed by Hofmann, Frenzel, et al. (9, 10) in graphite and graphite acid.

The purpose of the present work was to study swelling in connection with the problem of soil structure. This problem is not new in soil science. It is known that the swelling of soil acts as a factor in the formation of structure because of the pressure developing during the process. On the other hand swelling causes dispersion and pulverization. As the experiments described in this paper have shown, large structural aggregates are divided into smaller ones or are definitely disintegrated into the mechanical units of which they were composed. This process becomes comprehensible if the swelling soil aggregate is considered as an osmotic cell, the inner contents of which, as the water is absorbed, gradually liquefy.

Another important circumstance that should be noted is the change in porosity after absorption of water, this change being connected with swelling and directly related to the structure of the soil. A number of investigators [e.g., Terzaghi (25), Tiulin (27, 28)] have repeatedly indicated that a change in porosity takes place in swelling soil. The quantitative

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<sup>1</sup> The study of swelling was conducted in the laboratory of A. N. Sokolowsky, to whom I express my thanks.

side of this phenomenon, however, has remained unelucidated, though without this, many important agrotechnical problems can not be solved (e.g., non-capillary porosity or aeration).

#### ON SOIL SWELLING

"Swelling" is usually understood to include the capacity of certain organic and inorganic bodies to jelly after contact with certain liquids or with their vapor. This phenomenon is accompanied by an increase in the volume and weight of the swollen substance, due to absorption of the liquid, or by changes in other physical constants, e.g., viscosity, pressure, etc.

The process of absorption of a liquid by a solid does not seem to be uniform. Capillary imbibition distinctive of porous bodies must be differentiated from the phenomenon of swelling (18). Capillary imbibition presents an analogy to what is customarily called "intermicellary swelling" (considered as a capillary phenomenon), a process arising either between the micellae or on their surface. Water is attracted by the surface of crystallites, because of the bipolar action of the ions, and tends to disunite them. No change in the degree of dispersion occurs in this process.

The process of swelling is in its essence characterized by the change in properties inside the micellae which accompanies the volume increase (intramicellary swelling). Utilizing this notion, we can distinguish between the following types of swelling:

I. The swollen micella keeps its molecular bond intact. Roentgenographically, this kind of swelling finds expression in differences—though not sharp ones—of patterns. Almost all the interferences retain their positions, except those with a small angle of reflection [swelling of crystals (8)]. This type of swelling is explained by the osmotic action of the solution within the micella. The inside of the cell is transformed into a liquid, in which Brownian movement is observed (6). According to the osmotic theory a micella is a system consisting of an insoluble elastic wall and inner contents. The inner contents—the so-called "liquid phase"—are a solution of the "solid phase." This solution represents the "third component," which according to Northrop (17) causes the swelling. According to Mattson (4, 13, 14) osmotic swelling is explained by an atmosphere of dissociating ions surrounding the colloidal soil particle. Under Na saturation the number of dissociating ions reaches its maximum, and the swelling reaches its maximum as well.

II. The swelling is characterized by a complete reconstruction of the inner structure of the micella. Roentgenographically this is expressed by the appearance of a diagram of a new substance, as, for instance, was found in the swelling of ramie fibers in concentrated solutions of alkalis (12). This type of swelling is characterized by a strong increase in dispersion, influencing greatly the amount of water absorbed, because the number of water films around individual crystals rises sharply. The action of water on aggregates of crystals consists not only in their separation from one another but in the destruction of the micella itself. Between these extreme types of swelling intermediary stages can occur, the characteristic features of which are not explained as yet.

The structure of the substance exerts a complicated influence on swelling, as has been found for cellulose (7). Structure is important also in the swelling of argillaceous minerals; for instance, the crystalline network of kaolinite and of pyrophyllite is less sensitive to the action of water than that of montmorillonite (3). Soil structure too seems to exercise an influence on swelling, if the structural unit is regarded as a complex of osmotic cells, the disposition of which in respect to one another is very variable.

Proceeding from the foregoing we may consider that destruction of the soil structure, as well as unsettling of the natural disposition of the aggregates, must influence swelling to a high degree. This may be evolved from the fact that pulverized soil absorbs more water than soil that has been allowed to keep its natural structure. The methods for the determination of swelling are, however, mostly based on the application of pulverized substances.

The first experiments on the determination of swelling in substances approaching soil in their structure were made by Spring (24). This author investigated the absorption of water from the geologic point of view. He used for his experiments an apparatus for measuring the pressure arising after the imbibition of liquid by a sandy or argillaceous mass. This apparatus consisted of a container covered from below by a membrane and connected at the top with a manometer. The container was filled with the mass that was to be tested, and after its immersion in water the "suction force" of the mass was determined by reading the manometer. Later this method, slightly modified, was used by Joffe and McLean (11), by Freundlich and Sachs (5), and by Shaw (21).

Mattson (14) was the first to investigate the swelling of colloids isolated from soil and saturated with Na and Ca. It was found that swelling increases with the charge of the colloids. Anderson (1) investigated the swelling of colloids isolated from different soils and saturated with Ca, Mg, K, Na, and H cations. Substitution of a cation by another that raises the charge of the colloid particles increases the swelling. According to their influence on swelling the cations rank as follows:

$$\text{Na} > \text{K} > \text{Ca} = \text{Mg} > \text{H}.$$

A review of the existing theories for the explanation of swelling may be found in Pavlov's (19) work on the swelling of cellulose.

#### METHOD OF INVESTIGATION

The soil samples are taken in the form of clumps or clods without disturbing the structure. This method of taking samples is preferable to the one in which a cutting cylinder or a borer is used, because in the latter case a disruption of the structure occurs, which is particularly noticeable in compact soils. Borers can be used for sampling very loose soils, with no fear of disrupting the structure, as shown in an earlier publication (23). The use of cylindric containers for the measurement of swelling by measuring the changes of volume does not eliminate certain errors because the soil column in the cylinder dilates only along its vertical axis, and this vertical dilation does not compensate horizontal swelling. Thus, depending on the orientation of soil particles in pulverized soil, the latter may show a different volume increase, as has been repeatedly observed experimentally. The cause of this is that soil swelling is an anisotropic property, i.e., it proceeds along definite axes of the crystals, as has been demonstrated by Hofmann, et al. (8).

In studying the swelling of soil lumps it is necessary to protect them from deformation upon water absorption. It was found that this could be achieved by means of a collodion film, which lets the water through under negative pressure developed by the soil and is elastic enough not to hinder the increase of volume. A 4 per cent solution of collodion proved suitable for this purpose. The more viscous sorts of collodion produce the most durable films. The durability of the film depends also on the character of the surface of the sample. Collodion films on rough samples are always more lasting than on smooth ones.

The samples, varying in weight from 17 to 60 gm., were placed on small round stands made of fine copper wire netting and covered with a sheet of filter paper. These were put into a large flat-bottomed basin, filled with distilled water to a level a little above the wire netting of the stands and covered with

a lid to prevent evaporation. Another set of samples with a coating of collodion was similarly treated.

The samples were weighed every 24 hours for the determination of the absorption of water. It was found that equilibrium was reached in a minimum of 3 days. Water adhering to the interior side of the netting and of the clods was carefully sucked away by means of filter paper. Deformed clods were excluded. The results are shown in table 1.

These experiments show that water absorption is similar in both cases, i.e., that collodion films may be used in the same way as that usually employed in swelling experiments with porous clay plates easily permeable to water. Evidently the pressure of swelling is so great that the rôle of the elastic resistance of the collodion is negligible in comparison with it.

TABLE 1  
*Water absorption by soil*

SOIL CLOUDS WITHOUT FILMS													
	1	2	3	4	5	6	7	8	9	10	11	12	13
Air-dry weight, gm. ....	40.17	41.44	38.79	41.86	32.01	36.5	48.7	64.0	37.43	29.17	36.3	35.20	29.73
Water absorbed, per cent. ....	33.2	39.2	34.5	38.2	34.9	36.8	39.6	41.2	39.9	41.1	39.3	38.6	37.1
SOIL CLOUDS COVERED WITH COLLODION FILMS													
	1	2	3	4	5	6	7	8	9	10			
Air-dry weight, gm. ....	49.21	59.08	44.33	40.79	32.76	38.31	32.71	37.41	36.40	33.21			
Water absorbed, per cent. ....	35.7	34.8	35.7	36.5	37.06	39.9	37.2	36.9	37.6	41.1			

Average water absorption for soil clouds without films:

$$M_1 = 37.97 \pm 0.7.$$

Average water absorption for soil clouds covered with collodion films:

$$M_2 = 37.24 \pm 0.61.$$

The advantage of the collodion film method is that it allows samples of every form and size to be fixed without particular difficulty. The films must be, as far as possible, of uniform thickness. If one cracks in drying, the damaged places are to be covered with collodion with the aid of a small brush or a glass rod. If a sample cracks during the swelling experiment it must be discarded.

The best method for determining the volume of a swollen sample is the hydrostatic one, i.e., by weighing the sample in the air and under water. After this the sample is dried at room temperature to an air-dry condition. As the process is reversible the volume of dry soil may be determined on the same sample with the aid of mercury (22). The absorption of water is determined with precision by the difference in weight, the error not exceeding 0.01 gm. The weight of the film is taken into account.

The amount of water absorbed ( $K_{\Delta}$ ) may be expressed in percentages of the volume according to the following formula:

$$K_{\Delta} = \frac{\alpha}{v_{\Delta}} .100 \quad (A)$$

in which  $\alpha$  stands for the volume of absorbed water and  $V_{\Delta}$  for that of the swollen soil.

#### CHANGES IN POROSITY AFTER SWELLING

The distortion of the results in the determination of capillary and non-capillary porosity is due to the changes taking place in the porosity of soil after absorption of water and the volume increase accompanying this process, as compared with the porosity of the same soil in air-dry condition. It has been repeatedly indicated that water is distributed over a greater volume of soil and that swelling is underestimated (27). But no quantitative solution of this problem had been found.

When soil absorbs water, accompanied by a swelling of its volume, porosity experiences a change. The quantitative side of this phenomenon may be expressed as follows:

The mass of the soil  $m$  remaining unchanged, its volume  $V$ , corresponding to its air-dry condition, changes in swelling to  $V_{\Delta}$ . Hence the volume weight of swollen soil  $d_{\Delta}$  will be:

$$d_{\Delta} = \frac{m}{V_{\Delta}} \quad (B)$$

The changed total porosity of the swollen soil  $P_{\Delta}$  may be expressed

$$P_{\Delta} = \frac{d - d_{\Delta}}{d} .100 \quad (C)$$

by analogy to

$$P = \frac{d - d_1}{100} .100 \quad (D)$$

where  $d$  is the specific weight of the solid phase of soil,  $d_1$  the volume weight of dry soil. From equations A, B, and C it follows that if  $d_1 \geq d_{\Delta}$  the total porosity of swollen soil  $P_{\Delta} \leq P$ , the total porosity of soil in its air-dry condition. Thus from formula C the change in porosity at any water content may be found: one needs only to measure the corresponding volume of the soil.

The relative change in porosity after water absorption may be found from:

$$\Delta = \frac{P_{\Delta} - P}{P} .100 \quad (E)$$

If  $P_{\Delta} = P$ , water is absorbed by the soil without any change in porosity. This is observed chiefly in the case of sandy and loamy fine sandy soils. A special interest is attached to absorption of water in the case of clayey and loamy soils, if it is considered from the point of view of the structural changes arising in the soil in the course of swelling.

As mentioned, two kinds of water absorption occur. The first is capillary imbibition, in which water is absorbed by the pores of the soil or by its inter-particle spaces and does not penetrate into the structural cell. No change of the degree of dispersion occurs in this case. The second case—swelling proper—is characterized by the disruption of the soil structure accompanying water absorption. This process is very complicated, but an idea of it can be formed from the following:

Soil structure consists of aggregates, the inner contents of which are chemically inactive material. It is the so-called skeleton of soil, composed of mechanical elements. The formation of aggregates is associated with the cementing properties of the soil colloids. The cause of the swelling of such a system may be represented in different ways, as follows:

1. As a result of the electrolytic dissociation of the molecules of the colloid films, the water penetrates through the film inside through the aggregates, thus causing its destruction.<sup>2</sup>

Thus, swelling and the breaking up of structural aggregates go hand in hand.

2. The water is attracted by the surface of the crystallites of the colloid film as a result of the bipolar action of its molecules. The electrostatic repulsion of the ultra-crystals having charges of the same sign increases the thickness of the water film surrounding the ultra-crystals and the distance between them becomes greater; this leads to the weakening of adhesion and to the breaking up of the structural cell of the soil.

The dependence and the ratio of the capillary absorbed water to the total porosity may be found by dividing (A) by (C):

$$R_{\Delta} = \frac{K_{\Delta}}{P_{\Delta}} \cdot 100. \quad (F)$$

This ratio expresses capillary porosity in percentages of the total porosity,  $P_{\Delta}$ , which may be considered as the value of the relative absorption of water. The quantity  $R_{\Delta}$  represents the capillary porosity when the soil is saturated with water.

Absorption of an inactive (non-polar) liquid  $K$  by the same soil will be expressed by

$$K = \frac{a}{V} \cdot 100 \quad (G)$$

where  $a$  is the volume of the inactive liquid absorbed and  $V$  is the volume of soil in air-dry condition.

<sup>2</sup> Compare with Ramann's "The process of swelling takes place in the presence of reversible colloids in the soil" (20, p. 327).

The quantity  $K$  expresses the capillary porosity of soil in air-dry condition, i.e., the unchanged capillary porosity of soil. The relative value of this porosity is

$$R = \frac{K}{P} \cdot 100 \quad (H)$$

If we compare the absorption of water with that of some inactive liquid, which causes no swelling of the soil, then, according to (H), with  $K_\Delta = K$  we have capillary absorption of water by the soil pores (capillary imbibition) without damage to the aggregate condition of soil. The process of capillary absorption is not, however, limited to this relation. Capillary imbibition may be accompanied by an increase in the volume of the absorbing soil. Then  $K_\Delta > K$ , i.e., water absorption is greater than the absorption of the inactive liquid. However, in this case the difference between  $K_\Delta$  and  $K$  is only seeming. If we correct the value of the water absorption  $K_\Delta$  according to the increase in the soil's volume,  $K_\Delta$  becomes equal to  $K$ . The corresponding correction is

$$r = \frac{K_\Delta \cdot Q}{100}, \quad (I)$$

where  $Q$  is the value of the volume increase. Thus if we have

$$K_\Delta - r = K$$

we have only capillary imbibition.

The volume increase of the soil that has absorbed water is determined from  $V_\Delta$  and  $V$  according to

$$Q = \frac{V_\Delta - V}{V} \cdot 100 \quad (J)$$

In all cases, when  $K_\Delta - r > K$ , there is swelling, accompanied by a change in the soil structure.

The quantitative expression of these changes may be obtained from the very simple ratio

$$S = \frac{K}{K_\Delta - r} \quad (K)$$

It follows from the ratio (K), that when  $S = 1$  there is no destruction of the aggregates. In all other cases this value will be smaller the greater is the destruction. The ratio  $S$  may be used as the index of the change in the degree of dispersion arising with the absorption of water by soil. It may be regarded as a quantity characterizing the stability of the corresponding soil structure. Therefore  $S$  acquires the meaning of a "structure coefficient."

From this point of view the usually accepted notion of "swelling water" (20)



may be substituted by a new one. We may consider "swelling water" as that part of the total amount of water absorbed which causes the complex changes in the colloid condition of soil, brings about the destruction of its structure, and leads to an increase in its degree of dispersion.

The value of "swelling water" may be calculated from

$$(K_{\Delta} - r) - K = Wq \quad (L)$$

i.e., on the basis of distinguishing sharply between soil swelling and the process of capillary imbibition. Therefore the value of "swelling water" characterizes the essence of the process of swelling better than the so-called "volume of swelling." It is quite possible that "swelling water" may serve as measure of the hydrophylic properties of soil, but in this case the question remains unanswered as to which of the components is dissolved in which: the dispersion medium (water) in the dispersion phase (soil colloids), or vice versa.

#### EXPERIMENTAL

The liquids used in the absorption experiments were water, paraffine, machine oil, and others as shown later. Each experiment was repeated several times. For the water absorption experiments all the samples were coated with a collodion film. In the oil absorption experiments samples of loose soils only were supplied with such films, as otherwise it would be impossible to work with them without fear of their breaking up. Preliminary tests with oil absorption, similarly to those with water absorption, showed no difference between samples with or without films.

The choice of the soil samples was based partly on their textural characteristics (loamy sands and loams) and partly on their alkalinity (solonetz), because it was presumed that in dependence on these the swelling properties of soil would reveal themselves to a greater or lesser degree of sharpness.

Tables 2 to 4 show the results of these experiments.

As may be seen from tables 2 and 3 fine loamy sand chernozem shows that absorption of water produces only a slight volume increase, on the average  $Q = 4.41$ . If we introduce the correction for the volume increase  $r = 1.37$ , we obtain for  $K_{\Delta} - r = 29.62$  and  $K = 29.47$ , almost equal values. Relative capillarity, i.e., the ratio of capillary porosity to total porosity, has also an equal value for the absorption of both water and oil:

$$\frac{K_{\Delta}}{P_{\Delta}} = \frac{K}{P} = 0.70$$

Swelling water  $Wq = 29.62 - 29.47 = 0.15$ , i.e., practically zero.

. All this leads to the conclusion that in this case a purely capillary absorption of water takes place and that no structural changes occur in the soil.

By means of water and oil absorption experiments, similar relations have been found to exist in loess-like loam from the Vinnitza Experimental Station. In this soil the average of nine water absorption determinations for  $Q = 12.5$ ,

TABLE 2

*Behavior of fine loamy sand chernozem from the terrace of Volchia River with water absorption*

SOIL SAMPLE	$m$	$V$	$V_{\Delta}$	$Q$	$K_{\Delta}$	$P_{\Delta}$
1	21.60	14.2	14.75	3.87	30.4	45.0
2	16.03	10.7	10.91	2.80	28.6	45.0
3	15.94	10.5	10.82	3.05	31.4	44.6
4	15.60	10.3	10.82	5.65	34.0	46.1
5	13.12	8.3	8.65	4.21	33.5	43.1
6	19.42	12.1	12.72	5.62	30.4	43.1
7	14.44	9.1	9.61	5.64	31.6	43.5
8	14.09	8.9	9.31	4.61	30.3	43.5
9	11.93	7.6	7.97	4.87	28.6	43.8
				$M = 4.41$ $\pm 0.34$	$M = 30.98$ $\pm 0.63$	$M = 44.2$

TABLE 3

*Behavior of fine loamy sand chernozem from the terrace of Volchia River with oil absorption*

SOIL SAMPLE	$m$	$V$	$K$	$P$
1	22.52	14.6	29.09	42.0
2	25.90	16.6	29.55	41.6
3	20.72	14.2	32.95	45.4
4	21.55	14.6	34.30	44.6
5	20.22	13.5	31.70	43.8
6	18.01	11.1	25.96	39.3
7	16.48	10.04	25.73	40.5
			$M = 29.47$ $\pm 1.65$	$M = 42.46$

TABLE 4

*Behavior of soils with benzene and isobutylic alcohol absorption*LOAMY SAND FROM THE TERRACE OF VOLCHIA RIVER  
WITH BENZENE ABSORPTIONLOESS-LIKE LOAM FROM THE VINNITZA EXPERIMENT  
STATION WITH ISOBUTYLIC ALCOHOL ABSORPTION

Soil sample	$m$	$V$	$K$	$P$	Soil sample	$m$	$V$	$K$	$P$
1	21.60	14.2	30.10	43.10	1	16.80	10.6	35.57	42.53
2	16.03	10.7	28.00	43.80	2	15.00	9.3	33.12	41.41
3	15.94	10.5	28.60	43.10	3	18.79	11.5	33.00	41.04
4	15.60	10.3	29.13	42.40	4	19.30	12.1	34.14	42.16
5	13.12	8.3	27.40	40.84	5	20.46	12.6	36.67	41.04
			$M = 28.65$ $\pm 0.46$	$M = 42.65$				$M = 34.50$	$M = 41.65$

$K_{\Delta} = 40.1$ , and  $P_{\Delta} = 49.62$ . In the case of oil absorption  $V = 12.5$ ,  $k = 34.33$ , and  $P = 43.58$ .

In comparison with loamy sand the volume increase in loess-like loam after water absorption is considerably greater and averages 12.5 per cent. After correcting this figure ( $r = 5.01$ ) we obtain a value almost equal to the original  $K$ , the same as in the case of loamy sand:  $K_{\Delta} - r = 35.09$  and  $K = 34.33$ .

Relative capillary porosity is equal here also:

$$\frac{K_{\Delta}}{P_{\Delta}} = \frac{K}{P} = 0.80.$$

Swelling water  $Wq = 35.09 - 34.33 = 0.76$ , i.e., practically zero, as in loamy sand. Therefore here also, in spite of the noticeable volume increase, reaching 12 per cent, only capillary absorption of water occurs.

To show that the nature of the inert liquid has no specific action on capillary absorption, parallel experiments with absorption of benzene and isobutylic alcohol were conducted. The results are shown in table 4.

These experiments show that, as could be foretold, the values of absorption proved the same for benzene and isobutylic alcohol; for instance, for loamy sand chernozem,  $K$  (oil) = 29.47 and  $K$  (benzene) = 28.65; for loess-like loam,  $K$  (oil) = 34.33 and  $K$  (isobutylic alcohol) = 34.50.

The experiments with the absorption of polar and nonpolar liquids by the soils mentioned *demonstrate with particular clearness* the fact that liquids with a different dielectric constant are absorbed by soil in equal amounts. This leads us to the conclusion that no changes connected with electrolytic dissociation of molecules take place at the interface of the phases  $\frac{\text{colloid substance}}{\text{water}}$ . The process of water absorption by these soils must be considered as a purely capillary phenomenon, which characterizes these soils as structureless, lacking entirely any elementary aggregate cells.

Diametrically opposite relations are found experimentally to exist in structural soils, as exemplified here by the data on (I) loamy chernozem from the terrace of Volchia River and (II) columnar alkali (solonetz) from the Khorlov district.

In I the values for water absorption were  $Q = 15.30$ ,  $K_{\Delta} = 42.36$ , and  $P_{\Delta} = 55.53$ ; for oil absorption  $K = 27.17$  and  $P = 47.34$ .

After introducing the correction for volume increase,  $r = 6.48$ , we obtain for  $K_{\Delta} - r$  (water absorption) the value of 35.88, i.e., 8.71 more than  $K$  (oil absorption). This difference constitutes the swelling water value [ $Wq = (K_{\Delta} - r) - K = 8.71$ ]. This is the water that is used up in the process of changes connected with the breaking up of the structural cell. Specific capillary porosity for water and oil is not the same

$$\frac{K_{\Delta}}{P_{\Delta}} = 0.76; \frac{K}{P} = 0.57.$$

For water this ratio is larger, as could be expected in connection with the breaking up of aggregates, because this break-up increases the number of capillary pores, both microscopic and submicroscopic. Corresponding to this, the structure coefficient  $S = 0.76$ .

Similar results, but even more striking, have been obtained also with II, the columnar alkali soil. In water absorption the value for  $Q = 21.66$ ,  $K_\Delta = 40.05$ , and  $P_\Delta = 46.44$ . In oil absorption  $K = 18.22$  and  $P = 33.64$ .

The correction for volume increase  $r = 8.67$ . Then  $K_\Delta - r = 31.38$ , which exceeds  $K = 18.22$  by 13.16, the value of swelling water. The values of the relative capillary porosities differ more than those of loamy chernozem:

$$\frac{K_\Delta}{P_\Delta} = 0.86 \text{ (water) and } \frac{K}{P} = 0.54 \text{ (oil).}$$

The structure index is here still smaller—

$$S = \frac{18.22}{31.38} = 0.58$$

And indeed columnar alkali soil differs from chernozem by a greater content of replaceable Na (its cation exchange capacity being 17.9 m.e.; Ca, 10.95 m.e.; Mg, 2.8 m.e.; and Na, 4.45 m.e.).

The results of the experiments on water and oil absorption are summarized in figure 1.

It may be seen from this graph how greatly the values of  $Wq$  ("swelling water"),  $\frac{K_\Delta}{P_\Delta}$  (water), and  $\frac{K}{P}$  (oil) differ from one another by the character of the curves for structural and structureless soils. Although the values of "swelling water"  $Wq$  and "relative capillarity"  $\frac{K_\Delta}{P_\Delta}$  (water) and  $\frac{K}{P}$  (oil)—do not differ in structureless soils (loamy sand chernozem and loess-like loam), a sharp divergence is observed in structural ones. In the latter, capillarity as determined by water absorption always exceeds that determined by oil absorption. In this case the results of our experiments coincide with those of the experiments on the absorption of xylol, the amount of which necessary for filling up the capillary spaces is always less than that of water, as has been shown by Tiulin (27).

The characteristic feature of structureless soils is that their  $Wq$ —swelling water value—is always practically zero. For structural soils the value of  $Wq$  will be the greater the greater is the breaking up of their structure.

#### DETERMINATION OF NON-CAPILLARY POROSITY

It is interesting that in structureless soils—in our experiments in loamy sand chernozem and loess-like loam—for which water absorption is a purely capillary process, the difference (i.e., non-capillary porosity) between  $P_\Delta$  and  $K_\Delta$  and  $P$  and  $K$  has the same value (table 5):

$$n = P - K = P_\Delta - K_\Delta \quad (M)$$

where  $n$  stands for non-capillary porosity;  $K$  for the volume of the capillaries as determined by absorption of oil or of some other inactive liquid (or what is the same thing—the capillary porosity of air-dry soil);  $K_{\Delta}$ , for the volume of capillaries as determined by absorption of water;  $P$ , for the total porosity of air-dry soil;  $P_{\Delta}$ , for the total porosity of soil after water absorption.

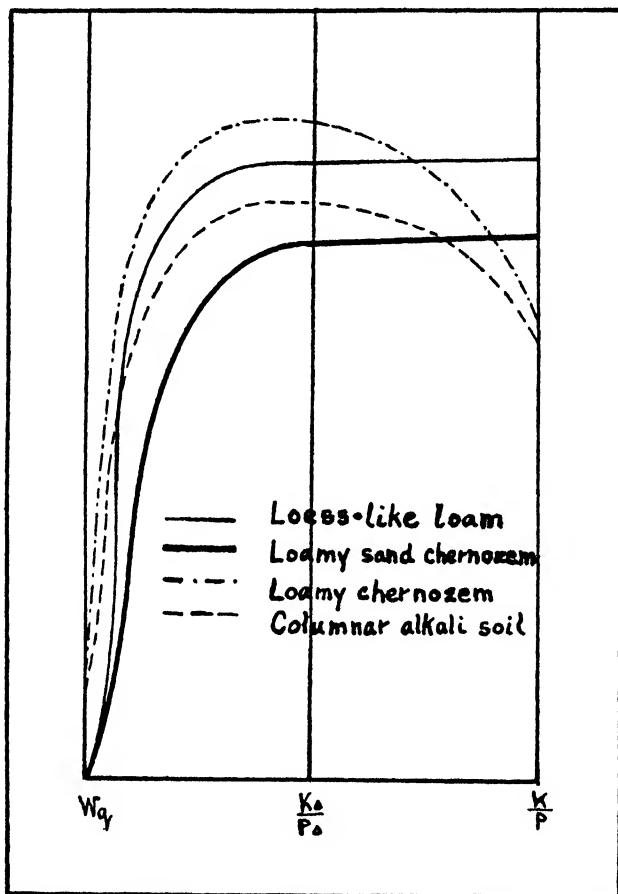


FIG. 1. "SWELLING WATER" ( $W_q$ ) AND "RELATIVE CAPILLARITY"— $\frac{K_{\Delta}}{P_{\Delta}}$  (WATER);  $\frac{K}{P}$  (OIL)

For structural soils the relation between capillaries and non-capillary spaces acquires another significance. It is necessary to take into account that the disruption of the structural (aggregate) cell is tied up with the dielectric constant of water. As a result of this, its penetration into the cell and the process of electrolytic dissociation associated with it will differ sharply from the penetration of a non-polar liquid. It must be assumed that the latter is absolutely incapable of penetrating into submicroscopic pores, which are accessible only

to water. Therefore, absorption of a non-polar liquid by a structural soil characterizes the "inter-particle" capillarity, i.e., the pores lying between the structural aggregates, but not the "submicroscopic" capillarity, i.e., the pores lying inside the cells. In structural soils absorption of non-polar liquids is always less, and the difference between  $P$  (total porosity) and  $K$  (oil absorption) does not represent a value characterizing non-capillary porosity. Non-capillary porosity computed in this way is greater than the real value because it includes the submicroscopic porosity. Submicroscopic pores may be calculated from formula  $L$ . They correspond to the volume of "swelling water," i.e., that water which has the property of penetrating inside the structural cell. Thus, formula  $M$  acquires the following form for structural soils:

$$n = P - K - Wq = P_{\Delta} - (K_{\Delta} - r) - Wq \quad (.12)$$

In this formula  $r$  stands for the correction for volume increase. The results are summarized in table 5.

TABLE 5  
*Non-capillary porosity of soils*

SOIL	$P_{\Delta}$	$K_{\Delta}$	$P$	$K$	$P_{\Delta} - K_{\Delta}$	$P - K$	$K_{\Delta} - r$	$Wq$	$P - K - Wq$	$P_{\Delta} - (K_{\Delta} - r) - Wq$
1. Loamy sand chernozem from Volchia River....	44.2	30.98	42.46	29.47	13.22	12.99	—	0	—	—
2. Loess-like loam from Vinnitza Experiment Station	49.92	40.10	43.58	34.33	9.82	9.25	—	0	—	—
3. Loamy chernozem from Volchia River.....	55.53	42.36	47.34	27.17	—	—	35.88	8.71	11.46	10.94
4. Columnar alkali soil from Khorlov district.....	46.44	40.05	33.64	18.22	—	—	31.38	13.16	2.26	1.90

The possibility of determining the "submicroscopic" porosity may form the basis for a differential analysis of porosity into non-capillary porosity, capillary porosity, and submicroscopic porosity. The latter is a specific indication of the process developing at the interface of the phases  $\frac{\text{colloid substance}}{\text{water}}$  and characterizes the desintegration of the structural cell.

#### AGGREGATE ANALYSIS, SWELLING WATER, AND INDEX OF TEXTURE STABILITY

Determinations of the amount of "waterproof" aggregates were conducted by the method of aggregate analysis elaborated by Tiulin (26). The amount of waterproof aggregates for the soils studied is compared with that already obtained for the same values of "swelling water" ( $Wq$ ) and texture index ( $S$ ) in table 6.

As may be seen from table 6 the results obtained by both methods coincide well. The amount of waterproof aggregates in loamy sand chernozem and in loess-like loam is practically zero, as the insignificant quantity of waterproof aggregates remaining on the sieve consists of coprolites of worms and of aggregates tied together by small roots of plants. The swelling water value for these soils is also zero, which permits classification of these soils as structureless.

TABLE 6  
*Aggregate analysis, swelling water ( $Wq$ ), and index ( $S$ )*

SOIL	AMOUNT OF WATER-PROOF AGGREGATES (PER CENT OF TOTAL)	$Wq$	$S$
1. Loamy sand chernozem.....	2.41	0.15	—
2. Loess-like loam.....	0.40	0.76	—
3. Loamy chernozem.....	28.46	8.7	0.76
4. Columnar alkali soil.....	12.66	13.7	0.58

TABLE 7  
*The influence of size and form of soil sample on oil absorption*

SOIL	HEIGHT OF SAMPLE	$m$	AMOUNT OF OIL ABSORBED PER 100 CC. OF SOIL	$P$
	mm.		gm.	
Loamy sand chernozem from Volchia River...	35.0	27.23	36.70	42.0
	60.0	45.65	37.00	42.0
	60.0	52.77	35.45	41.22
	80.0	76.27	32.60	42.0
	80.0	81.57	33.20	42.0
Loess-like loam from Vinnitza Experiment Station.....	35.0	19.15	33.10	43.65
	35.0	24.20	32.60	43.65
	60.0	48.68	32.50	43.65
	60.0	51.68	32.90	43.65
	90.0	94.22	30.80	43.65
	90.0	117.00	30.30	43.65

In loamy chernozem and columnar alkali soil the amount of waterproof aggregates is in inverse ratio to "swelling water" ( $Wq$ ) and in direct ratio to the "texture index" ( $S$ ). The greater is the value of  $Wq$  the less is the amount of waterproof elements. The value of the index  $S$  decreases with the decrease in the amount of waterproof aggregates.

#### SOME DETAILS OF THE ANALYTICAL METHODS AND THE SIZE OF SAMPLES

The size and form of samples used for porosity analyses is very important. Most non-polar liquids have a considerably lower capillary constant than

water. It is necessary, therefore, to pay attention to *the height of the sample* and to the character of the porosity of the soil. For finely porous soils the height of samples, it was found, must not exceed 60 mm. Experiments with saturation with oil of samples of different heights were arranged. The results are shown in table 7. Loamy sand chernozem and loess-like loam samples were taken for these experiments.

As may be seen from table 7, the sample 35 mm. in height does not differ in its absorption of oil from the sample 60 mm. high. Small deviations are due to the variations in porosity, as may be seen from the last column of the table.

Oil determinations must be executed with certain care. After absorption, the sample is left for some time on the sheet of filter paper soaked in oil. The excess of oil adhering to the surface of the sample trickles down on the paper.

TABLE 8  
*Influence of size of soil sample on water absorption*

SOIL SAMPLE	m	V	V <sub>Δ</sub>	Q	K <sub>Δ</sub>	m	V	V <sub>Δ</sub>	Q	K <sub>Δ</sub>
1	202.02	141.41	163.08	15.68	39.63	47.70	33.33	39.08	17.25	42.09
2	196.01	136.77	156.83	14.66	41.41	40.38	28.17	32.68	16.13	42.23
3	244.55	170.64	194.58	14.02	41.39	41.49	28.95	33.70	16.64	42.31
4	215.12	150.11	175.90	16.11	43.29	38.88	27.13	31.21	15.04	45.91
5	222.18	155.03	180.77	16.60	38.58	45.37	31.65	36.38	15.00	41.18
6	236.00	164.68	191.40	16.23	40.91	46.85	32.69	37.37	14.31	41.58
7	208.14	145.23	170.78	17.58	42.37	45.37	31.65	36.47	15.22	42.09
8	238.94	166.73	191.78	15.04	39.69	37.77	26.34	30.80	16.93	41.98
				M <sub>1</sub> = 15.74 ±0.40	M <sub>1</sub> = 40.9 ±0.54				M <sub>2</sub> = 15.82 ±0.38	M <sub>2</sub> = 42.42 ±0.51

The data on swelling and water absorption for larger and smaller samples given in table 8 show the influence of the size of sample on the accuracy of the determinations.

From comparison of the average values of  $Q$  (volume increase), it follows that:  $M_2 - M_1 = 0.08 \pm 0.17$ . From comparison of  $K_\Delta$  (water absorption),  $M_2 - M_1 = 1.51 \pm 0.74$ . Thus no differences have been observed. A certain increase in the water absorption ( $K_\Delta$ ) and swelling ( $Q$ ) values is noticeable in smaller samples. This is in accordance with the "Bodenkörperregel" theory and with the experiments on the influence of the mass of swelling gelatine on its volume (13).

#### SUMMARY

The study of the phenomena of swelling of soil that keeps its natural structural condition allows investigation of the problem of soil structure from a new point of view.



A method has been elaborated, and a form for a quantitative expression of the porosity changes caused by absorption of water by soil, evolved.

Definitions of the "index of texture stability" ( $S$ ) and of "swelling water" ( $Wq$ ), founded on the sharp difference between capillary imbibition and swelling proper, are introduced.

Swelling water may be considered as the form of expressing the hydrophillic property of soil.

Soil swelling is considered as a process of changes arising in the properties of soil at the interface  $\frac{\text{soil colloids}}{\text{water}}$ . This process leads to a change in the structure of soil and to an increase in its degree of dispersion.

A quantitative expression of this process is possible in the form of "swelling water," which represents a more characteristic indicator of swelling than the increase of volume.

A method is proposed for a differential analysis of soil porosity, providing for its subdivision into non-capillary, capillary, and submicroscopic porosity.

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## BOOK REVIEWS

*Weeds.* By WALTER CONRAD MUENSCHER. The Macmillan Company, New York, 1935. Pp. xxi + 577, figs. 123, tables 11. Price \$6.00.

The channels of trade are also in part those of weed distribution, and, since the coming of the white man to North America, many new species of plants have been brought in. Some of them are listed among the most valuable of our staple crops. Inadvertently, there came also plants objectionable or undesirable, and they are commonly designated as *weeds*. The present work is a valuable treatise on the subject of weeds. It sums up our present-day knowledge and offers to the student and the general reader information in very accessible form.

As is noted in the preface: "Part I is devoted to a consideration of those characteristics and habits of weeds by which they affect other plants or interfere with man's activities, and also to the methods employed for their eradication and control. In Part II is brought together data concerning the names, duration, reproduction, dissemination, habitat, range, source, recognition and control of the commonest weeds of the northern United States and Canada. Particular emphasis has been given to identification and control."

There are four chapters in Part I, dealing, respectively, with the dissemination and importance of weeds, weeds of special habitats, the control of weeds, and chemical weed control. Part II contains a list of weeds arranged according to family, together with key. A key to the groups and species of weeds is also given.

Altogether, the author has succeeded in rendering a distinct service to the farmer and to the specialist in commerce and agricultural science, on whom he must depend for the profitable conduct of his business.

*Suppression of Weeds by Fertilizers and Chemicals.* By H. C. LONG. H. C. Long, Hook, England, 1934. Price 2s. Pp. 57, figs. 12.

The preparation of this booklet was prompted by the need for a popular presentation of the status of weed-control by means of fertilizers and chemicals. As is noted in the foreword: "For many years now the destruction of weeds by the application of some chemical has been occupying the attention of investigators, and one material after another has been recommended for practical use. Little by little information has accumulated, and as some of the methods have proved their value the time is opportune for their serious consideration by farmers." The foreword by Sir Daniel Hall and the author's preface are followed by eleven chapters, designated as follows: Losses due to Weed Infestation; Effect of Fertilizers in Reducing Weeds; The Use of Lime; Sulphate of

Ammonia and Nitrate of Soda; Calcium Cyanamide; Sulphuric Acid; Sulphate of Copper and Iron; Finely Powdered Kainit; Sodium and Potassium Chlorates; Compounds of Arsenic; and Miscellaneous. There is also a list of weed names.

*The Algae and Their Life Relations.* By JOSEPHINE E. TILDEN. The University of Minnesota Press, Minneapolis, Minnesota, 1935. Pp. xii + 550, figs. 257. Price \$5.00.

The author is to be commended for the effective treatment of a most interesting subject. She has placed under obligation to herself many teachers, research workers, and students in the plant and soil sciences. As is indicated by the author:

The book is primarily a text for advanced students in botany. It is designed as a guide to the student, a supplement to lectures and laboratory work, and a reference book for experienced investigators. The algal plants are described in the earlier chapters. Their relations to other plants and animals, their distribution and economic uses, are discussed in later sections.

Every aspect of phycology is given attention. Terms have been simplified and reduced in number, and a helpful series of life cycle diagrams has been included. The volume also contains a discussion of the economic significance of algae; their uses as food for men and animals, their effects on water supply, and their value as fertilizer.

*Der Einfluss der Düngung auf den Pflanzenbestand des Dauergrünlandes.* By K. PLOTZE. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, Germany, 1935. Pp. 80.

The growing interest in erosion control and soil conservation has forced the recognition upon us that the best method of controlling erosion must be based on the maintenance of a proper plant cover. Many million acres of agricultural land have been damaged because of the removal of the topsoil by way of gullying or sheet erosion. It is more widely recognized now than heretofore that, because of its topography, much of our present crop land must be maintained under a forest or grass cover if the surface soil is to be protected from further injury by erosion. Quite logically, the increasing acreage under grass almost automatically compels us to inquire concerning methods of grassland management. The author of this book discusses facts of interest to farmers, investigators, and teachers.

The book was written from the point of view of the needs of German agriculture. The author notes in his preface that Germany is compelled to increase her resources of forage crops in order that it may be less dependent on imports of certain agricultural commodities. The subjects which he deals with, in a rather popular way, consist of a general statement, the influence of lime on the botanical composition of permanent meadows, the influence of farmyard manure, the influence of potassium and phosphorus fertilization, the influence of kainit and calcium cyanamid for the control of weeds, and the influence of nitrogen fertilizer. The list of references consists of 131 titles.

*Fertilization of the Canadian Prairies.* The Consolidated Mining and Smelting Company of Canada, Ltd., Calgary, Canada. Pp. 48.

The booklet is made up of a number of short articles dealing with one or another phase of Canadian prairies and their fertilization. It is stated in the foreword that

Successful fertilization of the Canadian prairie soils began four years ago, when the combined fertilizer project between the Dominion and Provincial Governments and leading industrial concerns was instigated and phosphate fertilizers were first applied by drilling in with the seed of cereal crops.

This booklet contains statements from many of the leading investigators in the Dominion and Provincial Governments, Universities and Agricultural Colleges, who were closely allied with the prosecution of the project. Also a few letters from some of the many actual farm users, together with certain economic data from actual large farmer operators.

*Die Bindung der Phosphorsäure im Erdboden. II. Untersuchungen aus West-norwegen.* By TORBJØRN GAARDER and O. GRAHL-NIELSEN. A. S. John Griegs Boktrykkeri, Bergen, Norway, 1935. Pp. 107, figs. 34.

This is the second part of a report on the fixation of phosphoric acid in soils. The first part appeared under the same title at Bergen, Norway, in 1930. It dealt specifically with the solubility of phosphoric acid in water solutions, with electrolytes with varying pH value and cation content.

The present volume records investigations made in Western Norway. The contents of the publication are arranged under the headings: Introduction; Discussion of the Soil Samples Investigated; Analytical Methods Employed; Discussion. There is also a résumé in Norwegian.

*Handbuch der Ernährung der landwirtschaftlichen Nutzpflanzen.* By J. BECKER-DILLINGEN. Paul Parey, Berlin, 1934. Pp. VIII + 523, figs. 124, plates 11. Price RM 19.60.

In preparing this book, the author wished to provide helpful data not only for the practical farmer, but also for the teacher and student. The major divisions of the book are designated, respectively, "General Considerations of Plant Nutrition," "The Soil as a Culture Medium of Plants," "Fertilizers and Fertilization," and "The Fertilization of Certain Economic Plants."

Consideration is given by the author in the first part to such questions as seed germination, the synthesis of plant products, and the destructive metabolism in plants. In the second section of the book the author deals with soils and soil science, soil formation, soil physics, soil chemistry, soil biology, and soil classification. In the third part consideration is given to animal manures and organic waste products. The fourth section treats of the fertilization of cereals, legumes, root crops, oil-bearing plants, fiber crops, commercial crops like tobacco and hops, forage crops, and meadows and forests. Numerous foot notes, adequate illustrations, and a satisfactory index add to the usefulness of the book.

*Der Speisewert der Kartoffel.* By KARL RATHSACK. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, Germany, 1935. Pp. 139, figs. 19, tables 49.

The growing of potatoes in Germany and in some of the other European countries is intended to cover a wider range of use than is true of the United States. As the author notes in his preface: "We differentiate our potato varieties in accordance with their particular fitness for manufacturing, human food, animal food or general purposes."

Since this treatise is devoted to the edible qualities of potatoes, emphasis is laid on cooking qualities, the firmness of the tubers and cooking quality as related to chemical composition, the relation of cooking quality to the length of the storage period, the changes which occur in the cooking of potatoes, the distribution of the chemical constituents within the tuber, and the chemical composition and taste. There are a list of references containing 96 titles and an author index.

*Pflanzliche Konstitutionslehre.* By F. MERKENSCHLAGER and M. KLINKOWSKI. Paul Parey, Berlin, Germany, 1933. Pp. 74, illus. 85. Price RM 7.50.

The authors approach their subject largely from the point of view of the ecologist. As is indicated by the table of contents, consideration is given to plants derived from humid environments and those derived from arid environments. In the first group are included potatoes, serradella, oats, and buckwheat. There are crops of a transient character, such as lupines and rye. The plants originating in arid environments include mustard, sugar beets, wheat, alfalfa, and barley.

The pamphlet is well illustrated, and the subject matter is presented in an interesting way. There is a bibliography attached to each of the major groups discussed.

*Die quantitative Spektralanalyse der Elemente.* By HENRIK LUNDEGÄRDH. Gustav Fischer, Jena, Germany, 1929 and 1934. Part I, pp. vi + 155, illus. 43, tables 13. Price RM 13.50. Part II, pp. viii + 124, illus. 39, tables 3. Price RM 20.

The first part of the report was published in 1929, and the second, five years later. As stated by the author, in the first part of the report, the quantitative methods for the spectrum analysis of elements were considered in their application to biological, agricultural, chemical, and mineralogical problems. It is noted in the second part that the author has improved the methods and indicated practical procedures for the carrying out of the analyses in the fields of biology, medicine, agricultural chemistry, and mining.

The contents of the first part of the report are indicated under the topics: Introduction; Quantitative spectrum analysis as based on the flame spectrum; Quantitative analysis as based on the spark spectrum; and Views on the application of quantitative spectrum analysis of the elements.

In the second part of the treatise, the major topics dealt with are designated

as Index; Quantitative emissions of spectrography, and the photoelectric measurements of spectrum lines, gas analysis, practical suggestions.

The work is splendidly illustrated, and the subject matter is well arranged. The author has made a substantial and valuable contribution on the subject of the quantitative aspects of spectrum analysis.

*Popoff's Quantitative Analysis* Third Edition. Revised by MURRAY J. RICE and WARREN P. CORTELYOU. P. Blakiston's Son & Co., Inc., Philadelphia, Pa., 1935. Pp. xxii + 555, figs. 75.

Extensive changes have been made in the book for the sake of bringing it in line with present-day studies of quantitative analysis. In the preface to the third edition, we are told that "One who is familiar with the second edition of this book may feel, on first consideration, that little of the original material has been preserved. A detailed examination will show otherwise. Dr. Popoff's original idea that 'quantitative analysis affords an excellent approach to theoretical chemistry and to skill and precision in experimental work' has been preserved and, it is hoped, emphasized. However, much of the material has been rewritten and nearly all of it rearranged in the belief that the new presentation will simplify the work of the student and of the teacher."

The preface to the third edition is followed by that to the first edition, and this, in turn, is followed by a foreword to the instructor and a foreword to the student. Part I of Book I contains 8 chapters; Part II, 7 chapters; and Part III, 9 chapters. Book II is made up of two parts, containing, respectively, 8 and 12 chapters. The appendix includes a glossary and table of abbreviations; references to analytical chemistry; how to use logarithms; how to use cologarithms; answers to problems; antilogarithms; four-place logarithms table.

Teachers and students will both find it to their interest to examine the book and to utilize it as a valuable addition to other teaching material in quantitative analysis.

*Annual Review of Biochemistry*. Vol. IV. Edited by JAMES MURRAY LUCK. Annual Review of Biochemistry, Ltd., Stanford University P.O., California, 1935. Pp. vii + 639. Price \$5.00.

The rapidly increasing number of workers in the general field of biochemistry and their efforts to keep themselves informed on the progress in their chosen field make the *Annual Review of Biochemistry* very helpful. In the preface to the present volume, the Editorial Committee notes: "In the introductions to the three preceding volumes we have described the circumstances leading to the inception of the *Review* and the considerations which seemed of major importance in the development of editorial policy. Most noteworthy are the unabated growth in the field as a whole, the ever-rising tide of published work urgently in need of integration and synthesis, the dramatic suddenness with which new discoveries illuminate old and vexing problems, and the tendency of other once-active phases to become quiescent."



They note, further: "In accordance with the policy of giving due recognition to fields of immediate interest, we are glad to include within the present volume reviews on choline and allied compounds by Professor Gaddum, on the biochemistry of malignant diseases by Mrs. Holmes, and on plant hormones by Professor Thimann. Reviews on soil bacteriology, biochemistry in relation to selected problems in medicine, and on the application of X-ray methods to the elucidation of the structure of compounds of biochemical interest will appear in Volume V (1936)."

The topics dealt with in the Review include: "Permeability" by M. H. Jacobs; "Biological Oxidations and Reductions" by R. Sonderhoff; "Enzymes" by J. B. Sumner; "The Chemistry of the Carbohydrates and the Glycosides" by J. C. Irvine and G. J. Robertson; "The Chemistry of the Acyclic Constituents of Natural Fats and Oils" by E. Chargaff; "The Chemistry of the Proteins and Amino Acids" by E. J. Cohn; "The Chemistry and Metabolism of the Compounds of Sulfur" by H. B. Lewis; "The Chemistry and Metabolism of the Nucleic Acids, Purines and Pyrimidines" by L. R. Cerecedo; "Carbohydrate Metabolism" by C. F. Cori and G. T. Cori; "Fat Metabolism" by C. Artom; "The Metabolism of Amino Acids and Proteins" by Y. Kotake; "The Metabolism of Creatine and Creatinine" by W. C. Rose; "Detoxication Mechanisms" by B. Harrow and C. P. Sherwin; "The Hormones" by B. A. Houssay, V. Deulofeu, and A. D. Marenzi; "Choline and Allied Substances" by J. H. Gaddum; "Vitamins" by L. J. Harris; "Nutrition" by S. Brody; "The Chemistry of Muscle" by P. Eggleton; "The Metabolism of Brain and Nerve" by E. G. Holmes; "Chemical Embryology" by J. Needham; "The Biochemistry of Malignant Disease" by B. Holmes; "Plant Pigments" by R. Kuhn; "The Alkaloids" by R. Robinson; "The Mineral Nutrition of Plants" by F. C. Steward; "Growth Substances in Plants" by K. V. Thimann; "Immunochemistry" by M. Heidelberger; "The Chemistry of Bacteria" by M. Stephenson; and "Index."

*Soils of Northern and Northwestern China.* By JAMES THORP and K. C. HOU.

Soil Bulletin No. 12, The National Geological Survey of China, Peiping, China, 1935. Pp. 154.

Much progress has been made within the past five years in the classification and mapping of the soils of China. The senior author, on leave from the Bureau of Chemistry and Soils of the United States Department of Agriculture, has helped the Chinese students to utilize the methods developed and perfected in the United States for the classification and mapping of soils. The late Dr. C. F. Marbut, Chief of Soil Classification and Mapping of the Bureau of Chemistry and Soils, was at the time of his death at Harbin on his way to China, after the sessions of the Third International Congress of Soil Science at Oxford, to join his fellow worker. His death is a loss to soil science.

The present treatise is made up of seven chapters, entitled, respectively: "Areas Investigated," "Soils and Crops," "Soils and Their Interpretation,"

"Agricultural Industries," "Irrigation, Alkali and Drainage," "General Recommendations," and "Soil Erosion and its Control." There is also a foreword, a summary, and a list of references and notes on the soils of the Salachi Irrigation Project in relation to reclamation.

The material is well arranged, and the authors are to be particularly commended for the quality of the maps, the illustrations, and the list of references. This report is particularly valuable and significant at this time in view of the more intimate coöperation among members of the International Society of Soil Science toward the completing of an authoritative map of the great soil regions of the world.

*The Struggle for Existence.* By G. F. GAUSE. The Williams & Wilkins Company, Baltimore, Md., 1934. Pp. ix + 163, figs. 41, tables 9.

In his foreword to the book, Dr. Raymond Pearl, of Johns Hopkins University, says: "The author of the present treatise, Dr. G. F. Gause . . . makes in this book an important contribution to the literature of evolution." Elsewhere, Dr. Pearl notes the growing interest in the subject. "This renewed and effective activity," he says, "seems to be due primarily to two things: first, the recrudescence of general interest in the problems of population, with the accompanying recognition that population problems are basically biological problems; and, second, the realization that the struggle for existence and natural selection are matters concerning the *dynamics of populations*, birth rates, death rates, interactions of mixed populations, etc."

Dr. Pearl notes again that the author "breaks new ground in this book. It will cause discussion, and some will disagree with its methods and conclusions, but no biologist who desires to know what the pioneers on the frontiers of knowledge are doing and thinking can afford not to read it."

The six chapters in the book deal, respectively, with "The Problem," "The Struggle for Existence in Natural Conditions," "The Struggle for Existence from the Point of View of the Mathematicians," "On the Mechanism of Competition on Yeast Cells," "Competition for Common Food in Protozoa," and "The Destruction of One Species by Another." There are two appendixes, a bibliography of 138 titles, and an index.

*Peasant Europe.* By H. HESSELL TILTMAN. Jarrolds Publishers, London, 1934. Pp. 282, illus. 47, map 1.

The keynote struck by the author in his foreword is a rather striking one. He says: "Western Europe, preoccupied with the problems of international relations, industry, and the future of armaments, is sometimes in danger of overlooking the fact that more than half the entire population of that Continent is composed of peasants. The immense territories of this hundred millions of cultivators (outside the frontiers of the U. S. S. R.), whose bent backs till the soil of the ocean of peasant-lands, stretch from the Black Sea to the Baltic, forming a natural barrier between East and West. The peoples who inhabit

that land of farmsteads—Poles, Ukrainians, Czechs, Slovaks, Hungarians, Southern Slavs, and the rest—together represent the largest single unit in Europe, split by artificial political walls, but united by the bonds of common interests and, in war or peace, usually a common fate. Those peasant territories remain to-day almost virgin soil for the world's manufacturers, populated by millions of potential customers clad in home-made clothing and living on the produce of their soil."

The contents of the book include a foreword and 18 chapters, designated as follows: I. The Other Half of Europe; II. Austria: Gateway to the Peasant Lands; III. The Kingdom of Serbs, Croats and Slovenes; IV. Croatia's Fight for Justice; V. The New Bulgaria Emerges; VI. Bulgaria To-day; VII. Bulgarian Interlude; VIII. Will "Greater Rumania" Achieve Greatness?; IX. Bessarabia: A Study in Decay; X. Bukovina—and its Peoples; XI. A Peasant Speaks; XII. Hungary: A Nation with a Grievance; XIII. Poland and its Peasants; XIV. A Nation Nobody Knows; XV. The Ukrainians Live On; XVI. Czechoslovakia: A Successful Experiment; XVII. The World Depression and the Peasant; XVIII. The Peasants Look at the Future; and Index.

The book makes interesting reading and is to be commended to the general reader as well as to the statesman and economist.

*The Agricultural Fair.* By WAYNE CALDWELL NEELY. Columbia University Press, New York, N. Y., 1935. Pp. xxii + 313, illus. 9.

To one familiar with the evolution of American agriculture, there is much of compelling interest in *The Agricultural Fair*. The organization and the procedures of agricultural fairs are a part of the inheritance from the Old World. The agricultural fair still serves as an important educational factor in this and other countries. As is noted by the editors' foreword: "No institution, perhaps, has exerted greater influence upon American rural life than the agricultural fair. Yet the story of its Old-World origin, its transfer to this side of the Atlantic, and its many-sided development in the United States has long awaited the pen of some one who was competent to trace its history and to evaluate its social significance. Fortunately Professor Neely, who essayed this double task, was admirably fitted by temperament and by training to undertake it."

Aside from the editors' foreword and the preface, there are four parts, dealing, respectively, with "Early History and Types of Fairs"; "The Evolution of the Agricultural Fair in America"; "The Functional Aspects of the Agricultural Fair"; and "Conclusion." The book contains also an extensive bibliography and an index.

*Plants and Human Economics.* By RONALD GOOD. Cambridge University Press, London, 1933. Pp. xii + 202, maps 8. Price \$1.75.

The following may be noted from the preface: "My object," the author says, "has therefore been to combine in small compass and, it is hoped, in

reasonable and readable fashion, not only the botanical facts but also, and frequently more important, the historical and economic facts required to give to those who begin the scientific study of botany an adequate humanistic background of reality to their subject. I have tried to give them the evidence that the science of plants is something more than a mere mental discipline."

The thirteen chapters in the book are designated, respectively, I. Introductory; II. The Nature and Sources of Food; III. The Life of the Green Plant; IV. Factors limiting Agricultural Production; V. Science and Agriculture; VI. Cereals and Pulses; VII. Vegetables: Salad Plants: Fruits; VIII. Beverages: Sugar and Starch: Oils and Fats: Spices; IX. Timber, Coal and Petroleum; X. Rubber: Resins, Balsams and Gums: Tans and Dyes: Fibres; XI. Alcohol: Drugs: Fodders: Miscellaneous; XII. The Useful Products of the Lower Plants: Concluding Notes on Vegetable Products; XIII. The Economic Botany of Great Britain. The reader will find at the end of the book a selected reference list, an appendix containing scientific and English names of commercial plants arranged in systematic order, and an index.

*World Sugar Production and Consumption.* By C. J. ROBERTSON. John Bale, Sons & Danielsson, Ltd., London, 1934. Pp. vi + 142. Price 5s.

Sugar is one of the major human foods. Sugar cane and sugar beets—to say nothing of other plant species—are the sources of this important carbohydrate. The primary and secondary products of our sugar crops are of signal importance in their effect on the economic, social, and even political structure of many peoples.

The purpose of the author in preparing the book is explained in the preface. He says: "Exhaustive treatises on the technical aspects of sugar production have been written, dealing with cane or beet agriculture, with the factory processes in the production of raw sugar from cane or beet and with sugar refining. A book giving a comprehensive survey of the world's production of sugar from both cane and beet and with emphasis on the economic-geographical aspects has, however, been lacking."

The book contains thirteen chapters, designated as follows: I. An Outline of the World Trade in Sugar; II. General Conditions of Cane-Sugar Production; III. General Conditions of Beet-Sugar Production; IV. Cuba; V. Java; VI. India; VII. British Empire Producers Excluding India; VIII. United States Producers Including the Philippines; IX. Other Cane-Sugar Producing Countries; X. Europe and the Soviet Union; XI. The Refining Industry; XII. The Component Factors on Consumption; XIII. Prospects of Consumption and Production. There are also a bibliographical note and an index.

*Fifty-Two Years of Research, Observation and Publication 1877-1929.* By HENRY FAIRFIELD OSBORN. Charles Scribner's Sons, New York, 1930. Pp. xii + 160.

The author notes in his preface:

In these days of intensive specialization both in education and in research, it is interesting to review the activities of a half-century initiated in the older, broader and more extensive school and continued in the modern, narrower and more intensive school. It has been a great opportunity and privilege to receive inspiration in the spirit of one century and to be carried forward into the grander opportunities of another century.

One object of this volume is to show the *educational* value of the older school, chiefly in *fitting the mind to attack new problems from the old, broad point of view*. . . . Another object is to show that the broad education and the broad point of view never carry us beyond the lecture platform or beyond the reputation of learning and culture unless breadth is the useful threshold for depth. In other words, intensive research through thousands of intelligently directed observations enlightened from time to time by generalizations or hypotheses becomes the starting point for entirely new chains of observation purposely directed to convert a working hypothesis into a new principle or a new law.

The major divisions of the work deal with the publication of research, ideal methods of research, fellowship of research, research in the universities, and the impulse of research in geology and biology.

The reader will find much interest in this little book and will be impressed by the wide range of subjects dealt with by the author.

*Report for 1934 Rothamsted Experimental Station.* Harpenden, England.

Gibbs & Bamforth, Ltd., St. Albans, England. Pp. 259.

Every well-informed student of soils knows something about the contributions that have been made by the Rothamsted Experimental Station in the domain of soil and plant science. The present report is a most interesting record of progress. It contains information on the organization and staff of the station, the Woburn Experimental Farm, the Imperial Bureau of Soil Science, publications of the Rothamsted Experimental Station, and other matters of interest.

The director of the station, Sir John Russell, notes the additions to the physical resources of the institution and summarizes in condensed form the accomplishments in the various fields of research with which the station concerns itself. There are brief reports by the members of the staff responsible for major research projects or fields of activity. A list of publications and brief abstracts of these publications are given. Numerous tables and other statistical data are given which supplement those in earlier reports. The reader will be impressed with the wide range of scientific thought and interest shown by the director and his associates at Rothamsted.

*Transactions of the Third International Congress of Soil Science. Vol. I.*

Thomas Murby & Co., London, 1935. Pp. xii + 428.

The editorial committee notes in the preface that: "The papers are arranged primarily in order of Commissions—papers for Commission I preceding those for Commission II, and so on. For this purpose, the sub-Commission for Alkali Soils has been called Va, the sub-Commission for Forest Soils Vb, and

the sub-Commission for Peat Soils VIa. Within each Commission, the papers are grouped according to the different subjects for discussion, and within each group, alphabetically according to the authors' names. The groups of papers are arranged in the order in which they will be discussed by each Commission. Papers to be presented at joint sessions of two or more Commissions have been allocated to the Commissions to which they seemed most appropriate."

This volume of Transactions was made available for distribution prior to the opening of the sessions of the Congress at Oxford on July 29, and was used by the delegates and visitors at the Congress to facilitate their following the papers presented at the different sessions. The editorial committee is to be complimented for having done their work well.

The papers are grouped under the major topics. These are—Commission I. Soil Physics: "Water in Soil"; "Binding and Disruption Forces in Soil Structure"; "Mechanical Analysis and Field Texture"; and "Surface Phenomena in Soils." Commission II. Soil Chemistry: "Exchangeable Bases"; "The Soil Absorbing Complex"; "Method of Estimating Plant Nutrients in Soil"; "Analytical Methods"; and "Appendices." Commission III. Soil Microbiology: "Recent Ideas Concerning the Oxidation of Ammonia in Nature"; "The Physiology and Ecology of Nitrogen-Fixing Organisms"; "The Decomposition of Plant Materials and Soil Organic Matter"; "Methods of Estimating Plant Nutrients in Soil"; "Quantitative Investigations of the Microbiological Population of the Soil"; and "Miscellaneous." Commission IV. Soil Fertility: "Problems in Crop Production"; "The Nitrogen Supply of Crops"; "The Decomposition of Plant Materials and Soil Organic Matter"; "Methods of Estimating Plant Nutrients in Soil"; "The Importance of Deeper Horizons in Plant Nutrition"; and "Miscellaneous." Commission V. Soil Genesis, Morphology and Cartography: "Reports on Soil Maps"; "Principles and Methods of Land Classification"; "Soil Formation and Soil Types"; "The Composition and Classification of Soils"; and "Schemes of Soil Classification." Sub-Commission Va—Alkali Soils. Sub-Commission Vb—Forest Soils. Commission VI—Application of Soil Science to Land Amelioration: "Water in Soils"; "Drainage Investigations"; "Irrigation, Erosion"; and "The Movement of Salts in Soil." Sub-Commission VIa—Peat Soils.

*Transactions of the Third International Congress of Soil Science. Vol. II.*

Thomas Murby & Co., London, 1935. Pp. 194.

This volume, containing the plenary session papers and the presidential address, was made available prior to the adjournment of the sessions at Oxford. The title of Sir John Russell's presidential address was "The Place of Soil Science in Agriculture." The volume also contains papers not included in Volume I of the Transactions. There are three papers listed under Soil Physics; two, under Soil Chemistry; two, under Soil Microbiology; three under Soil Fertility; three under Soil Genesis, Morphology and Cartography; and two under Application of Soil Science to Land Amelioration.

*Report of the Iowa Twenty-Five Year Conservation Plan.* By JACOB L. CRANE, JR., and GEORGE WHEELER OLCUTT. Publication of The Iowa Board of Conservation and The Iowa Fish and Game Commission, 1933. Pp. xiii + 176, plates 53.

This report was sponsored jointly by the Iowa Board of Conservation and the Iowa Fish and Game Commission. The following is quoted from the foreword:

We believe that this report records the desire of the people of Iowa to exercise forethought through planning. Their first interest just now is to avoid wasting available funds. By establishing a long-term schedule of development on which every dollar spent will be well spent, an enormous economy is assured as compared with haphazard, uncorrelated conservation. The Plan is a device to get the people's money's worth in each phase of the work.

"The people of Iowa have for twenty years dreamed of the recovery, development and wise utilization of the woods, lands and waters from which the great wealth of the state is derived. Every element of the Conservation Plan is set forth in response to a strong demand from thousands or hundreds of thousands of citizens. This report is issued, therefore, to advise on the manner in which the things they want may be crystallized into a feasible, economic program which can be actually realized.

The contents of the volume are made up of a foreword, summary of recommendations, fourteen chapters, and a bibliography. The chapters are named, respectively: I. The Genesis of the Conservation Plan; II. The Conservation Plan Survey; III. The Historical Background; IV. Iowa and Its People; V. The Conservation of Iowa's Soil; VI. The Conservation of Iowa's Waters; VII. Woodland Conservation in Iowa; VIII. The Conservation of Wild Life; IX. The Conservation of Game; X. The Conservation of Iowa's Fisheries. Resource; XI. State Preserves and State Parks; XII. The Highways; XIII. Recapitulation—Unclassified Projects; and XIV. Fulfillment of the Conservation Plan.

The sponsors of the report have rendered a distinct and valuable service in having prepared and published a readable and attractively arranged statement.

*Price Indexes in China and Foreign Countries.* 1932. Ministry of Industries, Nanking, China. Pp. 247.

The report, published by the Ministry of Industries of Nanking, China, records in Part I index numbers of wholesale prices; index numbers of retail prices; index numbers of cost of living. Part II has to do with foreign countries. The topics considered are the comparison of index numbers of wholesale prices in Melbourne, Tokyo, London, New York, and Paris; index numbers of wholesale prices in foreign countries; index numbers of retail prices in foreign countries; index numbers of cost of living in foreign countries.

The headings of the titles are given both in English and in Chinese.

*An Annotated Bibliography of the Low Temperature Relations of Plants.* By RODNEY BEECHER HARVEY. Burgess Publishing Company, Minneapolis, Minn., 1935. Pp. 223.

The author of the Bibliography is professor of plant physiology, agricultural

botany, and horticulture at the Minnesota Agricultural College and Experiment Station, University of Minnesota. He has evidently made a very thorough-going search of the literature on low temperature relations of plants and has rendered a signal service to the students of the subject.

The author notes that: "the first typing of part of this bibliography was done in 1927. Copies of this list were deposited with the Low Temperature Research Station at Cambridge University, with the Institute für Pflanzenkrankheiten at Bonn, with Dr. N. A. Maximov at the Institute of Applied Botany at Leningrad, and with the Office of Forage Crops Investigations of the U. S. Department of Agriculture. Students at the University of Minnesota have had free use of the citations for more than ten years. Publication of the list is now made with the hope of decreasing the labor of reference to the literature."

JACOB G. LIPMAN.

*Kultura Bolot (Marsh and Swamp Culture)*. By B. D. ONOSHKO. Sel'khozgiz, Moscow, U. S. S. R., 1934. Pp. 1-574.

In this book the author reviews the results of investigations of the 32 Swamp Experiment Stations in the U. S. S. R., up to 1934. It is divided into five parts: I. The principles of agriculture on swamp soils, 9 chapters; II. Field crops for swam soils, 9 chapters; III. Meadow and pasture on swamp soils, 12 chapters; IV. Vegetable growing on swamp soils, 8 chapters; V. Miscellaneous agricultural plants on swamp soils. The book is profusely illustrated (145 plates) and has a list of close to 500 Russian references. It is to be regretted that the author failed to include at least the more important contributions of the Western European and American investigators on the subject. Even with this shortcoming it is a valuable contribution.

J. S. JOFFE.





# STUDIES OF THE ROOT NODULE ORGANISMS OF CERTAIN WILD LEGUMES<sup>1</sup>

MARIE ECKHARDT CONKLIN<sup>2</sup>

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The present investigation is concerned with a study of the root nodule bacteria of certain legumes which grow wild in Massachusetts, Connecticut, and New York and which may have an importance in natural green-manuring far greater than has been credited to them. Its purpose is to show, by a study of their morphological, cultural, and physiological characteristics, the relationship between the organisms isolated from different wild legumes, to characterize them so that they may be recognized by definite reactions, to establish their cross-inoculation position, and to show their relationship to other members of the inoculation group to which they may be found to belong.

The organisms were isolated from the following plants, the names, with two exceptions (6, 7), being taken from Gray's Manual (1908)<sup>3</sup>: *Amphicarpa bracteata* (L.) Fernald [*A. monoica* (L.) Ell.], *Baptisia tinctoria* (L.) R. Br., *Cassia nitilans* L., *Crotalaria sagittalis* L., *Desmodium paniculatum* (L.) DC., *Genista tinctoria* L., *Lathyrus latifolius* L., *Lathyrus japonicus*, Willd., var. *pellitus* [*L. maritimus* (L.) Bigel.], *Lespedeza hirta* (L.) Hornem., and *Lespedeza frutescens* (L.). Most of these plants are widely distributed in the eastern part of the United States. Although *Lathyrus latifolius* is not a native species, it is, according to Gray's Manual (1908), "frequently cultivated, and escaping to roadsides and thickets, Ct. to D. C." In addition, *Dolichos Lablab* L., an introduced form which is cultivated in Japan as a legume crop [Akemine (1931)], was used as a source of two cultures.

## HISTORICAL

Scattered references to the characteristics and cross-inoculation position of some of the wild legume organisms described in this paper occur in the literature and are reviewed in the monograph on the root nodule bacteria by Fred, Baldwin, and McCoy (8). The principal conclusions drawn from these previous studies are that *Amphicarpa bracteata* will not cross-

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<sup>3</sup> See note to "references."

inoculate with any other common legume; that *Baptisia tinctoria*, *Cassia nictitans*, and *Genista tinctoria*, on the basis of studies made with one strain of organism from each host, belong to the cowpea group, as do certain species of *Crotalaria*, *Desmodium*, *Lespedeza*, and possibly *Dolichos Lablab*; and that some species of *Lathyrus* belong in the pea group. The monotrichous flagellation, slow growth on ash agar, and inability to produce marked changes on litmus milk or to liquefy gelatin which have been noted for the organisms from *Baptisia tinctoria*, *Genista tinctoria*, and certain species of *Amphicarpa*, *Cassia*, *Desmodium*, and *Lespedeza*, and the peritrichous flagellation, rapid abundant growth on ash agar, and ability to change litmus milk which have been noted for the strains from certain species of *Lathyrus* are the chief physiological characteristics so far assigned to these organisms. Recently, Carroll (5) has placed *Desmodium triflora*, *Lespedeza sericea* and *L. stipulaceae* in the cowpea inoculation group, and *Lathyrus tingitanus* in the pea group and has noted fairly close protein kinship among fifteen species of *Crotalaria* and, in most cases, between the *Crotalaria* species and cowpea.

## MATERIAL AND METHODS

### *Cultural study*

Cultures were isolated from plants which Dr. Alice M. Ottley, of the Wellesley College Botany Department, and Dr. H. K. Svenson, of the Brooklyn Botanic Garden, very kindly identified.

Stock cultures were carried on yeast-extract mannitol agar to which Congo red, when making isolations, and brom thymol blue, when required to indicate hydrogen-ion concentration (9), were added.

Litmus milk, Endo's agar, and lead acetate agar were prepared from the "Difco" dehydrated product of Digestive Ferments Co., the milk being sterilized 10 minutes at 8 pounds' pressure for 3 consecutive days, and incubated for 1 week at 28°C. to test for sterility before using. Potato slants (9), neutral red glycerol ammonium phosphate solution (11), asparagine tyrosine agar made by adding 1.5 gm. of tyrosine to 1,000 cc. of asparagine mannitol medium (9), and standard beef-extract peptone gelatin sterilized for 10 minutes at 8 pounds' pressure on three consecutive days were also used.

Sugar media, with brom thymol blue indicator [Baldwin and Fred (1927)], were prepared from arabinose, glucose, fructose, lactose, maltose, sucrose, or raffinose and sterilized as in previous studies [Eckhardt, Baldwin, and Fred (1931)]. By the use of a standard method of sterilization, the results of the fermentations are comparable in spite of the slight destruction of certain sugars by heat. To discover the extent of this fixed error, growth and reaction on fructose agar prepared in the foregoing manner and prepared by adding the sugar solution, sterilized through a Berkefeld filter, to the previously sterilized nitrate agar were compared. In most cases growth was the same; in a few cases, slightly more abundant on the medium sterilized by filtration. The two media varied only slightly in their final fermentation reaction.

Dextrin; starch; the glucosides amygdalin, aesculin, and salicin; the alcohols dulcitol, erythritol, ethylene glycol, and glycerol; and the organic acid salts potassium oxalate, sodium succinate, and sodium urate were also used in 1 per cent concentration in the aforementioned basic medium. The fer-

mentation reactions produced by the organisms on the carbohydrate media were recorded as pH values for comparative purposes, but in interpreting these results, the limitations of the method must be considered.

Representative amino acids as the sole carbon and nitrogen supply were added to a mineral solution of the following composition:

Dibasic potassium phosphate.....	0.5 gm.
Magnesium sulfate.....	0.2 gm.
Sodium chloride.....	0.2 gm.
Distilled water.....	1,000.0 cc.
Brom thymol blue, 0.5 per cent alcoholic solution .....	5.0 cc.

To tubes containing the sterilized solution, 0.5 per cent alanine, cystine, leucine, tyrosine, or a mixture of these four, previously sterilized by filtration, or in the dry state at 15 pounds' pressure for 15 minutes, was added under sterile conditions. Since cystine and tyrosine are difficultly soluble in water, the precipitate made judgment of growth impossible, and observations of reaction changes were used in these cases to compare the growth activities of the organisms, although absence of any change did not necessarily indicate failure to grow. Other media containing 0.5 per cent alanine, leucine, or tyrosine, and 1.5 per cent agar to permit more accurate comparison of growth were prepared. A growth accessory factor isolated from cane sugar of retail commerce by the method of Allison and Hoover (1) was added to three sets of the amino acid agar in concentration of 0.008 per cent; of these, one set also contained 1.0 per cent mannitol, and a second, 0.05 per cent potassium nitrate.

Dye and salt sensitivity were tested by the plate streak method, using nitrate mannitol agar to which had been added varying concentrations of dibrom-oxymercuri fluorescein, or in which the amounts of sodium chloride, magnesium sulfate, and dibasic potassium phosphate were varied. Resulting growth was in each case compared with that of a control.

All cultures were incubated at 28°C., and transfers were always made from stock cultures. Many isolations were made from each host plant in an attempt to avoid generalizations without due consideration of possible strain variations.

#### *Microscopic study*

Gram or dilute carbol fuchsin stains were made from cultures 24 hours, 1 week, and 1 month old. Flagella stains (12) were made after transferring 24-hour agar streaks to sterile water and incubating for 3 days.

#### *Plant inoculation*

Bacteria-free seeds, often filed with a sterile file to hasten germination and then washed in mercuric chloride followed by sterile distilled water, were germinated and planted in sterile washed sea sand (found by repeated Kjeldahl determinations, modified to include nitrates, to be free from nitrogen) in

glazed or paraffin-coated jars or in Erlenmeyer flasks. Modified nitrogen-free Shive's or other standard nutrient solution was sterilized and used as needed. Contamination was guarded against by growing most of the plants in sterile glass cases or covering the pots with cellophane or ground cork and watering through a sterile tube. Six weeks' or 3 months' growth was required by many of the slow-growing wild legumes. Controls, uninoculated, were distributed among the inoculated plants and found to be nodule-free.

#### EXPERIMENTAL

All cultures were isolated and studied by the writer working at Wellesley College and Brooklyn Botanic Garden except those from *Lathyrus latifolius*, which were isolated and studied by Miss Lydia Kittell, under the direction of Dr. L. M. Snow at Wellesley College.

Since it was found, after cultural and cross-inoculation studies were made, that the organisms under investigation fell into two main groups, a characterization under this grouping follows. Due consideration should be given to the strain variations which are noted in the detailed tabulations.

Group 1 includes 20 cultures isolated from *Amphicarpa bracteata*, 24 from *Baptisia tinctoria*, 27 from *Cassia nictitans*, 5 from *Crotalaria sagittalis*, 10 from *Desmodium paniculatum*, 9 from *Genista tinctoria*, 3 from *Lespedeza hirta*, 2 from *L. frutescens*, and 2 from *Dolichos Lablab*. Group 2 includes 3 cultures isolated from *Lathyrus latifolius*, and 13 from *L. japonicus*.

#### *Characterization of Group 1*

The organisms of group 1 are fine, Gram-negative rods, bearing at the "corner" a single flagellum, and often swollen, vacuolated, and slightly irregular with age. On yeast-water mannitol agar they show rather slow, scant to moderate, white or colorless, smooth growth, without absorption of Congo red, and with an alkaline fermentation of mannitol, no growth or very scant white or creamy growth on potato slants, with substrate unchanged, and no visible change of litmus milk beyond an alkaline reaction in some cases after 2 weeks' to a month's incubation. No visible change is produced on neutral red glycerol ammonium phosphate solution; growth on gelatin is poor or lacking with no liquefaction within three months; no appreciable amount of tyrosinase is formed, although growth on asparagine tyrosine agar is good.

Growth of representative strains from *Amphicarpa*, *Baptisia*, *Cassia*, *Desmodium*, and *Lespedeza hirta* is fair on Endo's agar, and small amounts of aldehydes are formed; usually negligible amounts of  $H_2S$  are produced on lead acetate agar. Growth is inhibited by 1.0 to 2.0 per cent dibrom-oxymercuri fluorescein agar, sensitivity generally decreasing with 0.5, 0.1, 0.05, 0.025, and 0.01 per cent, and normal or increased growth resulting from the presence of 0.005 per cent of the dye. Complete inhibition of growth of most strains with 0.5, 1.0, and 2.0 per cent  $K_2HPO_4$ ; slight or no inhibition with 0.1 per cent  $NaCl$  or 1.0 per cent  $MgSO_4$ ; slight stimulation of some strains by 0.025

and 0.05 per cent NaCl or 0.1 and 0.5 per cent  $\text{MgSO}_4$ ; and more marked stimulation by 0.5 per cent NaCl, 0.05 per cent  $\text{MgSO}_4$ , or 0.1 per cent  $\text{K}_2\text{HPO}_4$  were noted on the media containing these salts.

Most strains produce an alkaline reaction on the sugar media, a few forming acid from arabinose. Arabinose, glucose, and fructose support a fair to abundant growth; lactose, maltose, sucrose, raffinose, and dextrin, a poor to moderate growth. Starch is not hydrolyzed, and growth is poor to fair. The glucosides amygdalin, aesculin, and salicin; the alcohols dulcitol, erythritol, glycerol, and glycol; and the organic acid salt sodium succinate are utilized for carbon and energy, and poor to moderate growth, usually with an alkaline fermentation, is produced. On potassium oxalate and sodium urate agars there is little or no growth. More detailed results of the fermentation tests are given in table 1.

Of the amino acids used in the media, leucine and, to a less extent, tyrosine are utilized as sources of both carbon and nitrogen by representative strains, and in most cases an alkaline fermentation of the former and a neutral or slightly acid or alkaline fermentation of the latter result. An alkaline reaction on cystine medium, complete inhibition of growth by a solution containing 0.5 per cent alanine, and impaired growth with 0.2 per cent alanine were noted. In a solution containing a mixture of alanine, cystine, leucine, and tyrosine of 0.5 per cent total concentration, far greater increase in pH occurs after growth than when any of these are present singly in 0.5 per cent concentration. A 30 to 125 per cent increase in the growth average on the amino acid media results when 0.008 per cent of an accessory growth factor is added, the percentage increase being most with alanine and least with leucine. Potassium nitrate as source of nitrogen produces little or no further increase in the average amount of growth, but mannitol as a carbon source produces an increase to an amount equal to or exceeding that of the control.

Results of all cross-inoculation tests are given in table 2. Every organism of this group produced small to medium, round or elongated nodules on the tap or lateral roots of the original host plant. Organisms from *Crotalaria*, *Lespedeza hirta*, and *L. frutescens* also produced nodules on cowpeas (*Vigna sinensis*), those from *Amphicarpa*, *Baptisia*, *Cassia*, *Genista*, and *Dolichos* on cowpeas and irregularly on soybeans (*Soja Max*), and those from *Desmodium* irregularly on both. Representative strains formed nodules on all other host plants of this group on which they were tested. Three strains from *Baptisia tinctoria* also formed nodules on *B. australis*; certain strains from *Genista tinctoria*, on *G. praecox* and *Cytisus hirsutus*; and the *Lespedeza* cultures, on *L. bicolor rosea*.

Although the foregoing characterization applies to most members of this group, variations among strains in both growth and reaction were of frequent occurrence, particularly on the carbohydrate and amino acid media. Other variations include the more capsulated, banded, slightly branched form of the *Amphicarpa* organisms; the slightly branched, coccoid, or club-shaped

TABLE 1  
Reactions (pH) produced by cultures on carbohydrate media

CARBOHYDRATE	SOURCE AND TOTAL NUMBER OF CULTURES												
	Group 1									Group 2			
	<i>Amphicarpa bracteata</i> —20	<i>Baptisia tinctoria</i> —24	<i>Cassia mitchiana</i> —27	<i>Crotalaria sagittalis</i> —5	<i>Desmodium paniculatum</i> —10	<i>Dolichos Lablab</i> —2	<i>Genista tinctoria</i> —9	<i>Lespedeza hirta</i> —3	<i>Lespedeza frutescens</i> —2	Variation	<i>Lathyrus latifolius</i> —3	<i>Lathyrus japonicus</i> —13	Variation
Arabinose	7.4 (11)*	7.4 (11)	7.4 (19)	7.6 (4)	7.4 (5)	7.6 (1)	6.2 (4)		7.6 (1)	6.0-7.6		6.0 (12)	6.0-6.2
Glucose	7.4 (18)	7.4 (12)	7.4 (21)	7.6 (4)	7.2 (4)	7.4 (1)	7.4 (9)	7.4 (2)	7.6 (1)	6.8-7.6	6.2 (3)	6.0 (12)	6.0-6.2
Fructose	7.4 (19)	7.4 (12)	7.4 (23)	7.4 (4)	7.4 (8)	7.4 (1)	7.4 (5)	7.4 (3)	7.2 (1)	6.8-7.6	6.6 (2)	6.2 (12)	6.0-6.6
Sucrose	7.0 (15)	7.2 (15)	7.2 (14)	7.4 (5)	7.4 (7)	7.4 (1)	7.4 (6)	7.0 (1)	7.4 (2)	6.4-7.6	6.2 (3)	6.2 (9)	6.0-6.2
Maltose	7.6 (17)	7.4 (13)	7.4 (24)	7.4 (5)	7.4 (10)	7.6 (1)	7.4 (8)	7.0 (3)	7.4 (2)	6.0-7.6	6.2 (3)	7.0 (9)	6.2-7.4
Lactose	7.4 (10)	7.4 (13)	7.0 (21)	7.2 (4)	7.2 (6)	7.0 (1)	7.4 (5)	7.4 (3)	7.4 (1)	6.6-7.4	6.0 (2)	6.2 (8)	6.0-6.4
Raffinose	7.4 (19)	7.4 (18)	7.2 (14)	7.6 (4)	7.4 (6)	7.2 (1)	7.4 (5)	7.4 (2)	7.4 (1)	7.0-7.6	7.6 (3)	7.0 (4)	6.2-7.6
Dextrin	7.4 (13)	7.2 (15)	7.2 (12)	7.6 (4)	7.4 (7)	7.6 (1)	7.4 (5)	7.2 (3)	6.8 (2)	6.8-7.6	7.4 (2)	7.0 (9)	7.0-7.6
Mannitol	7.4 (18)	7.4 (13)	7.4 (16)	7.6 (4)	7.6 (8)	7.0 (1)	7.6 (7)	7.4 (3)	7.2 (1)	6.6-7.6	6.2 (3)	6.0 (13)	6.0-6.2
Amygdalin	7.2 (2)	7.2 (3)	7.0 (1)	7.2 (2)	7.4 (2)	7.2 (1)	7.4 (2)		7.2 (1)	6.6-7.4		6.6 (3)	6.6
Aesculin	7.2 (2)	7.4 (2)	7.2 (1)	7.2 (2)	7.2 (3)	7.2 (1)	7.4 (2)		7.4 (1)	6.8-7.4		7.0 (2)	6.8-7.0
Salicin	7.2 (2)	7.2 (3)	7.2 (3)	7.2 (3)	7.2 (3)	7.0 (1)	7.2 (3)		7.2 (1)	6.8-7.2		6.2 (2)	6.0-6.2
Dulcitol	7.2 (2)	7.2 (2)	7.2 (2)	7.2 (1)	7.2 (3)	7.2 (1)	7.2 (3)		7.2 (1)	7.0-7.4		6.6 (2)	6.4-6.6
Erythritol	7.0 (3)	7.0 (2)	7.0 (2)	7.0 (2)	7.0 (2)	7.2 (1)	7.0 (3)		7.2 (1)	6.4-7.2		6.2 (2)	6.2-6.4
Glycerol	7.0 (2)	7.4 (3)	7.2 (3)	7.2 (2)	7.0 (2)	7.2 (1)	7.2 (2)		7.4 (1)	7.0-7.4		6.2 (1)	6.0-6.4
Glycol	7.2 (2)	7.2 (3)	7.2 (2)	7.2 (2)	7.4 (2)	7.4 (1)	7.2 (3)		7.2 (1)	6.8-7.4		7.0 (2)	7.0-7.2
Potassium oxalate	6.8 (3)	7.0 (3)	7.0 (3)	7.0 (3)	7.0 (3)	7.0 (2)	7.0 (2)		7.0 (2)	6.8-7.0		6.8 (2)	6.8-7.0
Sodium succinate	6.8 (3)	7.4 (2)	7.2 (2)	7.2 (2)	7.2 (3)	7.2 (2)	7.2 (2)		7.4 (1)	6.8-7.4		7.4 (2)	7.2-7.4
Sodium urate	7.0 (2)	7.0 (2)	7.2 (2)	7.0 (3)	7.0 (2)	7.2 (1)	7.0 (2)		6.8 (2)	6.8-7.2		7.2 (2)	7.0-7.2

\* Numbers in parentheses indicate the number of strains producing the particular reaction. When the reaction was not uniform throughout the agar, the pH of the upper half of the tube was recorded. Where pH values are given as 6.0 or 7.6, they indicate an acidity of 6.0 or more and an alkalinity of 7.6 or more, since these figures represent the limits of color change of the indicator.

Desmodium organisms; the more abundant growth of the *Amphicarpa* and *Dolichos* cultures, and the more rapid growth of the *Genista* cultures on yeast-water mannitol agar, and the neutral or acid reaction produced by a few strains from *Baptisia*, *Cassia*, *Dolichos*, and *Lespedeza frutescens* on this medium; the production of a serum zone on litmus milk by one strain from *Lespedeza frutescens*, two from *Amphicarpa*, and two from *Baptisia*, reduction of the litmus by these two *Baptisia* cultures and by three strains from *Cassia*, two of which also produce an acid reaction; the formation of a limited amount of tyrosinase by a few strains from *Amphicarpa* and *Baptisia*; and an acid reaction on both tyrosine and cystine media by the *Dolichos* cultures.

### *Characterization of Group 2*

The organisms of group 2 are short, often coccoid or enlarged, frequently branched, peritrichous, Gram-negative rods. They show a rapid, abundant, colorless, very gummy growth on yeast-water mannitol agar, with no absorption of Congo red, and an acid fermentation of mannitol; typical lack of or very scant, creamy growth on potato with the substrate unchanged, and a fair to moderate growth on asparagine tyrosine agar, with the production of tyrosinase negligible or entirely lacking.

All strains from *Lathyrus japonicus* produce a definitely alkaline reaction with partial to complete reduction and a half inch serum zone on litmus milk and no appreciable color change on neutral red glycerol ammonium phosphate solution. Their growth is completely inhibited by 1.0 or 2.0 per cent dibrom-oxymercuri fluorescein in the agar and impaired by as low as 0.005 per cent of the dye; completely inhibited by agar containing 0.5, 1.0, or 2.0 per cent  $K_2HPO_4$ ; unchanged or slightly inhibited with 0.1 per cent NaCl or 1.0 and 0.05 per cent  $MgSO_4$ ; unchanged or slightly stimulated by 0.025 and 0.05 per cent NaCl or 0.1 and 0.5 per cent  $MgSO_4$ ; and in some cases stimulated by 0.5 per cent NaCl or 1.0 per cent  $K_2HPO_4$ .

Organisms from *L. japonicus* produce an acid fermentation of all of the sugars except maltose, raffinose, and dextrin; poor growth on starch agar without hydrolysis; fair growth on fructose, raffinose, and dextrin; moderate growth on arabinose, glucose, lactose, and sucrose; and abundant growth on maltose agars. They produce a moderate growth and acid fermentation of the glucosides amygdalin and salicin; a neutral reaction on aesculin agar (neutral red indicator); moderate growth and acid fermentation of the alcohols dulcitol, erythritol, and glycerol; poor growth and alkaline reaction on glycol medium; fair growth and alkaline reaction on sodium succinate agar; and very poor growth with a neutral or alkaline reaction on potassium oxalate and sodium urate media.

Organisms from *Lathyrus latifolius* produce an acid reaction on all the sugar media except raffinose and dextrin, with poor growth and partial hydrolysis of starch reported. They show fair growth on raffinose and dextrin; moderate growth on maltose, lactose, and sucrose; and abundant growth on glucose and fructose agars.



TABLE 2  
Cross-inoculation results

HOST PLANT	TOTAL NUMBER OF ORGANISMS	ORIGINAL HOST	COWPEA	SOYBEAN	SWEET PEA	GARDEN PEA	SPRING VETCH	LUPIN	CLOVER	ALFALFA	GARDEN BEAN	<i>Gonista tinctoria</i>	<i>Gonista praecox</i>	<i>Baptisia tinctoria</i>	<i>Baptisia australis</i>	<i>Cassia nitilans</i>	<i>Cassia marylandica</i>	<i>Robinia pseudo-acacia</i>	<i>Cytisus hirsutus</i>	<i>Lathyrus maritimus</i>	<i>Laspedeza bicolor rosea</i>
Cowpea-soybean type: <i>Amphicarpa bracteata</i>	20	20+	8+	1± 2-	2-	1-	2-	2-	1-	2-	1-	1+		1+	3+		2-	2-		1-	
<i>Baptisia tinctoria</i>	24	24+	9+	4+																	
<i>Cassia nitilans</i>	27	27+	22+	4+				15-	14-	8-	9-	3+		2+			11-				
<i>Crotalaria sagittalis</i>	5	5+	5+	4-				4-	4-	2-	2-	2+		2+							
<i>Desmodium paniculatum</i>	10	10+	2+	5+																	
<i>Dolichos Lablab</i>	2	2+	2+	1+				1-	1-	2-	1-	1+		1+							

<i>Genista tinctoria</i>	9	9+	6+	1+														2+		2+
			1-	6-																
<i>Lespedeza hirta</i>	3	3+	2+																	2+
<i>Lespedeza frutescens</i>	2	2+	2+																	2+
Totals	102	102+	58+	15+	2-	13-	2-	22-	20-	14-	13-	7+	2+	6+	3+	1+	13-	2-	2+	1-
			6±	4±																
			12-	44-																
Pea type:																				
<i>Lathyrus latifolius</i>	3	3+			2+		3+													
					1±															
<i>Lathyrus japonicus</i>	13	13+			8+	1+	1+													
					2±	1±	5-													
					1-	9-			2-	2-	2-	1-		1-						
Totals	16	16+			10+	1+	4+		2-	2-	2-	1-		1-						
					3±	1±	5-													
					1-	9-														

\* The numbers refer to the number of strains of the organism which were tested, and the + and - to the resulting nodulation. Two or more pot each containing three to six plants, were inoculated with each strain.

The *L. japonicus* organisms utilize leucine and, to a less extent, tyrosine as sole carbon and nitrogen sources, producing an alkaline reaction in each case, show an alkaline reaction on cystine medium, are inhibited by a solution containing 0.5 per cent alanine, and show decreased growth with 0.2 per cent. There is no increase in pH after growth in a solution containing a mixture of amino acids over that occurring when these are present singly. Growth on amino acid agar is increased by the addition of 0.008 per cent accessory growth factor, shows little or no further increase on the addition of 0.05 per cent KNO<sub>3</sub>, but marked increase in growth and change of reaction from alkaline to strongly acid on the addition of 1.0 per cent mannitol.

All strains produced medium-sized, smooth, round to elongated nodules on the tap root or laterals near the tap root of the original host plant. Strains of the *L. japonicus* organism usually produced nodules on *L. odoratus*, rarely on *Pisum sativum* or *Vicia sativa*, and never on any other legume tested. Organisms from *L. latifolius* produced nodules on *Vicia villosa*, and irregularly on *L. odoratus*.

#### DISCUSSION

The foregoing characterization of group 1 agrees generally with the results of previous workers who found, in part, that the organisms from *Baptisia tinctoria*, *Genista tinctoria*, and certain species of *Amphicarpa*, *Cassia*, *Desmodium*, and *Lespedeza* were monotrichous, slow-growing rods, showing a thin, scant growth on ash agar, meager growth on potato, no liquefaction of gelatin, and no change on milk [Burrill and Hansen (1917), Löhnis and Hansen (1921), Shunk (1921), Müller and Stapp (1925)], and that the organism from a species of *Crotalaria* may pass through a cycle of short, motile rods, cocci, and banded rods [Gangulee (1926b)], whereas those from species of *Desmodium* are short rods showing very little branching or irregularities (10). Stapp (1923) reported a negative tyrosinase test on the one strain of *Genista tinctoria* organism which he studied, but nine strains of the organism were here found to produce no appreciable amount of tyrosinase. The alkaline (10 days) to neutral (2 weeks) fermentation of glucose and the neutral reaction on sucrose agar by a culture from a species of *Lespedeza* [Schönberg (1929)] are in agreement with the alkaline to neutral reactions resulting from growth of the *Lespedeza* cultures on these media in the present investigation.

The organisms from group 1 resemble those isolated from cowpea and soybean because of their monotrichous flagellation; swollen, vacuolated, unbranched bacteroids; slow growth of scant to moderate amount; inability to produce gelatinase or caseinase; and failure to grow well on potato [Burrill and Hansen (1917), Löhnis and Hansen (1921), Wright (1925a, b)]. In addition, they show marked similarity to both of these organisms in their ability to utilize the common monosaccharides, disaccharides, and trisaccharides, dextrin, certain glucosides, alcohols, and, to some extent, certain organic acid salts, producing, in most cases, an alkaline fermentation; in their more abundant growth and frequently acid reaction on a pentose sugar medium; and in

their failure to hydrolyze starch [Baldwin and Fred (1927), Wilson (1917), Walker and Brown (1930)]. The alkaline reaction produced by these cultures on litmus milk, usually accompanied by no other change, is also a characteristic of the cowpea and soybean organisms [Wright (1925a, b), Stevens (1925b)]. The cultures of group 1 resemble the cowpea organism and certain strains of the soybean organism in their failure to produce tyrosinase, although variations in this characteristic occur among soybean strains [(2), Stapp (1923)].

Further evidence of the relationship of the organisms of group 1 to the cowpea and soybean cultures was found in the cross-inoculation studies, in which certain strains from every host plant of this group formed nodules on cowpeas or soybeans, although irregularities in both of these crosses occurred.

Previous workers, on the basis of inoculation tests with one strain of the organism from *Baptisia tinctoria*, *Cassia nictitans*, and *Genista tinctoria*, included these legumes in the cowpea group [Burrill and Hansen (1917), Leonard (1923a), Walker (1928)] and also certain species of *Crotalaria*, *Desmodium*, and *Lespedeza*, as well as *Dolichos Lablab* (the latter having produced nodules when inoculated with the cowpea organism) [Gangulee (1926b), Richmond (1926a), Carroll (5), Burrill and Hansen (1917), Whiting and Hansen (1920), Löhnis and Leonard (1926), Pieters (1927)].

There is also some evidence in previous literature of the production of irregularities in nodulation of cowpeas by these organisms. For example, Koch and Butler (1918) reported negative results from inoculating cowpeas with *Lespedeza striata* cultures, since nodules were not formed every time the experiment was made, but positive results, though poor, from inoculating velvet bean, a member of the cowpea group. Cultures from *L. striata* and *Cassia chamaecrista* formed nodules on lima beans, which belong to the cowpea group, but in two cases results with the latter cross were negative the first time it was attempted [Whiting and Hansen (1920)]. Similarly, Burrill and Hansen (1917) found that *C. chamaecrista* organisms caused nodules on cowpeas but that in one case out of four a cowpea culture of proved nodule-forming ability failed to cause nodules on *C. chamaecrista*, and they also presented evidence, although they considered it inconclusive, that cultures from *Cassia nictitans* failed to produce nodules on *C. chamaecrista*, *C. medsegeri*, and *Vigna sinensis*.

Inoculation of soybeans by organisms from *Lespedeza striata* and *Desmodium tortuosum* [Leonard (1923a)] or by the organism from *Crotalaria juncea* [Gangulee (1926b)] has been reported as negative, but Leonard (1923a) stated that a strain isolated from *Cassia nictitans* formed nodules on both cowpea and soybean.

Bearing further on this question are the results of Leonard (1923a), Sears and Carroll (1927), Carroll (5), Walker and Brown (14), and others who have noted that organisms from cowpeas irregularly form nodules on soybeans and that the soybean organisms almost always produce nodules on cowpeas, thus suggesting a definite, though irregular relationship between these two cultures.

The present investigation on the cross-inoculation position of the legumes

of group 1 has brought to light so many irregularities that it is difficult to draw any clear-cut conclusions beyond the fact that the organisms from this group of legumes are related to one another and to both the cowpea and soybean organisms on the basis of their cultural characteristics and also of their cross-inoculation capacities. It has generally been considered that if an organism isolated from one legume will form nodules on another legume these two plants belong in the same inoculation group. Accordingly, *Crotalaria sagittalis* appears to belong to the cowpea group, since all strains produced nodules on cowpeas, and to belong in the same group with *Genista tinctoria* and *Baptisia tinctoria*, since it also formed nodules on these plants. Organisms from both *Genista tinctoria* and *Baptisia tinctoria* produced nodules on cowpeas; therefore, in spite of the fact that a few strains gave negative results on cowpeas in the tests made, their position in that group seems warranted, and a definite relationship to *Rhiz. japonicum* is indicated by their ability irregularly to form nodules on soybeans. *Amphicarpa bracteata*, although previously considered to belong to none of the known inoculation groups [Burrill and Hansen (1917)], *Dolichos Lablab*, *Lepedeza hirta*, and *L. frutescens* seem rightly to be members of the cowpea group, since all strains from these plants which were tested on cowpeas gave positive results and since the strains tested on *Baptisia tinctoria* and *Genista tinctoria* also produced nodules. Some positive and some negative results on soybeans with cultures from *Amphicarpa* and *Dolichos* are evidence of their irregular relation to *Rhiz. japonicum*. A strain of the *Dolichos* organism also formed nodules on *Cassia nictitans*, and cultures from the latter produced nodules on cowpeas, *Genista*, and *Baptisia*; hence, inclusion of *Cassia nictitans* in the same cowpea group seems justified. Irregularity in nodulation of soybeans is characteristic of the organism from *Cassia*, and, in addition, certain strains did not consistently form nodules on cowpeas. Their failure to form nodules on *Cassia marylandica* is supported by the observation of Leonard (1925b) that this species of *Cassia* bears no nodules. The organisms from *Desmodium paniculatum* showed irregularity in nodulation of both cowpeas and soybeans; hence the position of this legume in either group, as defined at present, is difficult to establish. The cultural reactions of the organism showed a relation to both cowpea and soybean organisms, as did the inoculation results, but the latter indicated a closer relation to *Rhiz. japonicum* than was noted with other members of group 1, because the percentage of positive results, the size and position of nodules, and the benefit to the host were greater on the soybeans.

The characterization previously drawn of group 2 is in agreement with that given by earlier workers of organisms from other species of *Lathyrus*, which they described as simple, peritrichous rods, often showing "X"- and "Y"-shaped, or large Indian-club, forms when old, and characterized by very fast, spreading growth, with abundant gum, on ash agar slants, a "good" growth on nitrogen-free maltose agar, and inability to produce tyrosinase [Schneider (1892), Beijerinck (1888), Burrill and Hansen (1917), Fred (1913), Shunk (1921), Harrison and Barlow (10), Stapp (1923)].

The organisms from group 2 resemble *Rhiz. leguminosarum* from *Pisum* and *Vicia* because of their peritrichous flagellation, branched bacteroids, abundant and rapid growth, inability to produce tyrosinase, capacity to form a serum zone and alkaline reaction on litmus milk and to utilize the common monosaccharides with the production of acid, certain disaccharides, trisaccharides, and alcohols with an acid or alkaline reaction, and certain glucosides, and, to some extent, organic acid salts with a usually alkaline reaction [Prucha (1915); Fred, Whiting, and Hastings (1926); Löhnis and Hansen (1919); Stevens (1925b)].

Cross-inoculation tests with cultures of group 2 confirmed their relationship with *Rhiz. leguminosarum*, but also brought out certain irregularities. *Lathyrus latifolius* has previously been placed in the pea inoculation group [Burrill and Hansen (1917)], and in these tests the *L. latifolius* organism formed nodules on both *L. odoratus* and *Vicia villosa*, although in the former case the nodules were small and few in number.

Other species of *Lathyrus* have been found to cross-inoculate within the genus and to form nodules on *Pisum* and *Vicia* [Simon (1914), Burrill and Hansen (1917), Carroll (5)], but *Lathyrus japonicus* has not previously been placed in any group. In the present investigation, the organisms from *L. japonicus* usually produced nodules on *L. odoratus*, but rarely on *Pisum sativum* or *Vicia sativa*. Since *L. odoratus* was placed in the pea group by Burrill and Hansen (1917), positive cross-inoculation with this plant would indicate that *L. japonicus* belongs to the same group. Variability among *L. japonicus* strains in producing nodules on pea and vetch suggest, as in the cowpea-soybean group, that the capacity to produce nodules on any legume other than the original host may not, under certain conditions, be a constant characteristic among all strains of a given organism, but rather a physiological strain characteristic, as suggested by Walker and Brown (14) in connection with the cowpea-soybean crosses. The irregularities in nodule production within the pea group, although not explained, are substantiated by evidence from previous reports of variability in cross-inoculation capacity among species of *Vicia* [Garman and Didlake (1914)], between *Pisum* and *Vicia* [Harrison and Barlow (10; 1907), Koch and Butler (1918)], and between *Vicia*, *Lathyrus*, and *Pisum* [Jardine (1924, 1926)], and of marked host specificity within the pea group [Helz, Baldwin, and Fred (1927)]. It is also noteworthy that *Vicia acutifolia* and *V. floridana* have recently been placed in a new inoculation group (5).

The other physiological characteristics of these two groups of organisms supplement the cultural methods already known for separating them, and in a few cases may be of use in distinguishing the organisms from different hosts within a given inoculation group.

None of the organisms caused any visible color change after 2 weeks' incubation in neutral red glycerol ammonium phosphate solution, and since several common contaminants decreased the pH with resulting change of indicator

color from red to yellow, a lack of such change may be a check on the purity of at least some of the Rhizobia.

Sensitivity to 0.005 per cent dibrom-oxymercuri fluorescein in mineral agar separated group 2, which showed greatly impaired growth, from group 1, which grew nearly as abundantly as the controls. This concentration of dye also caused marked stimulation in growth of the Desmodium cultures, which exceeded that of the controls and was thus a distinguishing characteristic. Strains from Baptisia failed to grow, those from Genista grew very slightly with 0.5 per cent dye concentration and other members of the group showed a fair growth. In this, as in all other experiments where growth was judged by comparison of visible bacterial mass, increase in apparent growth may be either cell increase or increased gum production.

Complete inhibition of growth by  $K_2HPO_4$  in concentration of 0.5 per cent or more, was the only result obtained from varying the concentrations of the salts used in standard nitrate mannitol agar which was consistent among all strains. An increase in growth over the control which was noted with most of the cultures on 0.1 per cent  $K_2HPO_4$  agar has been previously reported by Truesdell (1917) with the alfalfa organisms. Separation of groups 1 and 2 might be effected by the use of 0.05 per cent  $MgSO_4$  in the agar, since this caused a marked increase in the growth of most strains in group 1 and a slight decrease with the Lathyrus organisms.

On the sugar media, the organisms of group 1 usually resembled the cowpea and soybean cultures in growth and reaction, but a few exceptions must be noted. The organisms from Amphicarpa, Cassia, Desmodium, and *Lespedeza frutescens* generally produced an alkaline reaction in 2 weeks on arabinose agar, whereas the cowpea organisms produced acid in that period [Baldwin and Fred (1927)]. Most of the Cassia strains produced a less alkaline reaction on certain sugar media than did the cowpea organisms [Baldwin and Fred (1927)], and the cultures from Dolichos grew more rapidly than the other members of the group. These differences might be eliminated if a greater number of strains were studied. The cultures from *Lathyrus japonicus* and *L. latifolius* usually gave the same reactions on the sugar media as did *Rhiz. leguminosarum* [Baldwin and Fred (1927)], but on fructose, lactose, and sucrose agars more acid was formed by the beach pea than by the garden pea organisms, and on raffinose agar the reaction from the garden pea organism ranged from slightly alkaline to neutral, and that from the beach pea bacterium from moderately alkaline to moderately acid. *L. latifolius* strains gave the weakly acid fermentation of fructose and the alkaline fermentation of raffinose given by the garden pea organism, but resembled the strains from beach pea in producing slightly more acid on sucrose and considerably more acid on lactose than the garden pea cultures. They also produced slightly more acid from maltose than did those from *Pisum sativum* and considerably more than did the *L. japonicus* strains.

The cultures of group 1 gave an alkaline reaction on the glucoside media,

whereas the *L. japonicus* organisms produced an acid fermentation of salicin and amygdalin, thus separating the two groups and also distinguishing the latter from the pea organism, which showed a neutral reaction on these two media [Baldwin and Fred (1927)]. Apparent failure of the *Lathyrus* organisms to produce acid from aesculin may have been due to the use of neutral red in place of brom thymol blue indicator.

Growth of all the organisms on the media containing various alcohols was less abundant than with the glucosides except in the case of glycerol. The poor growth on dulcitol agar is interesting, since the isomeric hexahydroxy alcohol supports very good growth. The acid fermentation of glycerol by the *Lathyrus* organisms and the alkaline fermentation by the members of group 1 may separate these two groups.

Of the organic acids used as a source of carbon and energy, sodium urate and potassium oxalate supported practically no growth, the only indication of any metabolic activity being a slight increase in pH in some cases. The two groups reacted similarly on the organic acid media, and fair growth with an alkaline reaction on sodium succinate agar was characteristic of all but the *Amphicarpa* cultures, which displayed poor growth and a neutral reaction.

The growth of the *Rhizobia* on a mineral medium containing various amino acids show that these organisms, like certain others (3, 4, 13), are capable of utilizing particular amino acids for both their carbon and their nitrogen metabolism. Growth is usually accompanied by an increase in pH probably due to an accumulation of  $\text{NH}_3$  resulting from deamination. Failure to show much increase in pH on tyrosine solution is in accordance with the results of Pohlman (1931c), who found that *Rhiz. meliloti* and *Rhiz. japonicum* utilized tyrosine slightly as a source of nitrogen, but without the production of ammonia, and hence may not, in all cases, have indicated failure to grow. Blanchetière (4) found, by analysis for total and ammoniacal nitrogen, that amino acids which are not attacked singly by *Bact. fluorescens* are attacked in a mixture. The increase in pH produced in a solution containing a mixture of four amino acids, totaling 0.5 per cent concentration, was far greater than occurred in solutions containing any of the amino acids singly, in the case of all but the *Lespedeza*, *Dolichos*, and *Lathyrus* cultures, indicating a similar situation within the genus *Rhizobium*. Whether this increased alkalinity is due to increased growth previously limited by the absence of a specific amino acid essential either for growth or for the formation of an enzyme needed to break down other amino acids, or whether an unknown factor is responsible is a matter for conjecture at present. These results are given in table 3.

The addition of 0.008 per cent growth factor to the amino acid agar produced a marked increase in the visible bacterial mass. Since this amount of the factor is equivalent to less than 0.006 per cent carbohydrate, it seems probable that the increase resulted not from the small amount of carbon added, but from a definite stimulatory effect. The failure to show much further increase when 0.05 per cent  $\text{KNO}_3$  was added indicated that these amino acids



serve nearly as adequately for nitrogen metabolism as does potassium nitrate. One per cent mannitol, however, increased the bacterial mass on leucine and tyrosine media to an amount equal to or exceeding that of the control. The amino acid thus appears to be a less available source of carbon than the mannitol, but the improved conditions for growth brought about by the

TABLE 3

*Reactions produced by cultures on a mineral solution containing amino acids of 0.5 per cent total concentration*

SOURCE OF CULTURE	ALANINE*	CYSTINE	LEUCINE	TYROSINE	ALANINE, LEUCINE, CYSTINE, TYROSINE
	pH	pH	pH	pH	pH
<i>Amphicarpa bracteata</i>	7.0(2)† 6.8(1)	7.0(2) 7.2(1)	7.0(3)	6.8(3)	7.6(2) 7.4(1)
<i>Baptisia tinctoria</i>	7.0(2) 7.2(1)	6.8(2) 7.0(1)	7.2(3)	6.8(2) 6.6(1)	7.6(2) 7.4(1)
<i>Cassia nictitans</i>	7.0(2) 7.2(1)	6.6(2) 7.0(1)	7.2(2) 7.0(1)	6.8(3)	7.6(2) 7.4(1)
<i>Crotalaria sagittalis</i>	7.0(2) 7.2(1)	6.8(2) 6.6(1)	7.0(3)	6.6(1) 6.7(1) 6.8(1)	7.6(2) 7.4(1)
<i>Desmodium paniculatum</i>	7.0(2) 6.8(1)	7.0(2) 6.8(1)	7.0(3)	6.8(3)	7.6(2) 7.4(1)
<i>Dolichos Lablab</i>	7.4(1) 7.0(1)	6.0(2)	6.6(2)	6.2(1) 7.0(1)	6.8(1) 7.2(1)
<i>Genista tinctoria</i>	7.2(2) 7.0(1)	6.8(3)	7.0(2) 7.2(1)	6.8(2) 6.7(1)	7.6(3)
<i>Lespedeza frutescens</i>	7.0(2)	7.0(1) 7.2(1)	7.0(2)	6.8(1) 7.0(1)	6.8(2)
<i>Lathyrus japonicus</i>	7.0(3)	7.4(3)	7.0(3)	7.2(2) 7.0(1)	6.9(3)

\* 0.2 per cent.

† Numbers in parentheses indicate the number of strains producing the given reaction.

production of acid from mannitol simultaneously with the production of ammonia from the amino acid must not be disregarded. The average results from twenty-five representative strains from each group are presented in figure 1, based on four arbitrary units of visible bacterial mass.

In a solution containing 0.5 per cent alanine, growth of all cultures was inhibited; in 0.2 per cent concentration growth was not so abundant as the con-

tol, even when 1.0 per cent mannitol was present. This toxicity of alanine may be comparable to the results of Pohlman (1931c), who found that 0.05 to 0.1 per cent glycoll was toxic to certain strains of *Rhiz. japonicum*.

The very alkaline reaction produced by the *L. japonicus* strains on cystine solution appears to separate these organisms from those of group 1, and the change from scant growth with neutral or alkaline reaction on leucine or tyrosine agars, with or without the growth factor or nitrate, to abundant growth with strongly acid fermentation when mannitol is present is also char-

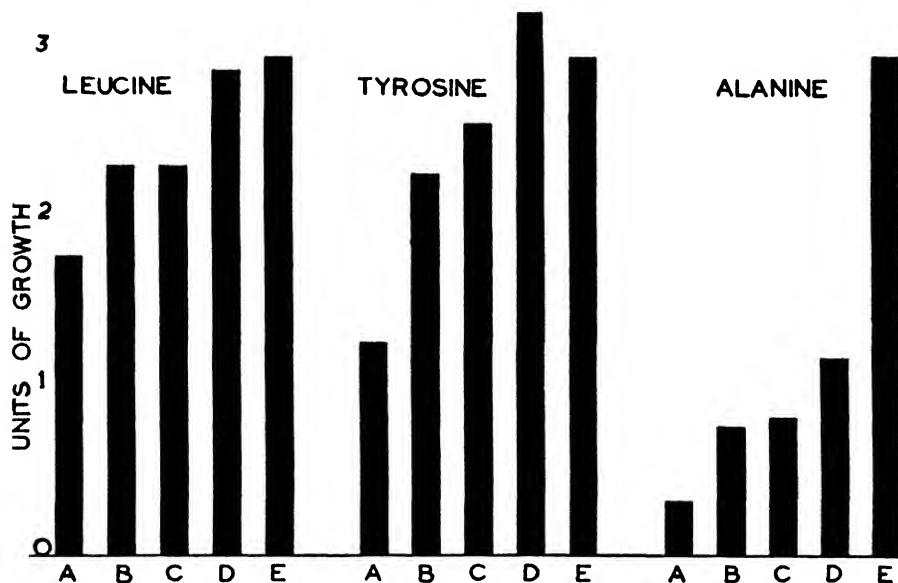


FIG. 1. AVERAGE BACTERIAL MASS PRODUCED BY 25 STRAINS OF WILD LEGUME ORGANISMS ON A MINERAL AGAR

The mineral agar contained:

- A. 0.5 per cent amino acid (0.2 per cent in the case of alanine).
- B. 0.5 per cent amino acid plus 0.008 per cent growth factor.
- C. 0.5 per cent amino acid plus 0.008 per cent growth factor plus 0.05 per cent potassium nitrate.
- D. 0.5 per cent amino acid plus 0.008 per cent growth factor plus 1.0 per cent mannitol.
- E. Control. Standard nitrate mannitol agar.

acteristic of the bacteria from *L. japonicus*. The strong production of acid on cystine solution and the slightly acid reaction on leucine solution might separate the *Dolichos* strains from other members of group 1. The *Baptisia* cultures showed some growth and an alkaline reaction on alanine agar without the growth factor, thus separating them, with two exceptions, from the other members of group 1 which were unable to grow on this medium.

The results of these cultural studies in most cases showed considerable variation among strains of an organism from any one host. This was particu-

larly true in both the rate and amount of growth of group 1 and in the dye and salt sensitivity of the organisms studied. On the carbohydrate media, variations in reaction between strains from the same host occurred occasionally, even between isolations from the same nodule. Although Dunham and Baldwin (1931), basing their distinction between strains on the grounds of inoculation effect, agglutination reaction, and certain cultural characteristics, concluded that in no case were there two strains in a single nodule, the differences in the nature of the colonies formed by different organisms which they noted may be comparable to the slight strain variations found here in fermentation reaction.

#### SUMMARY

Organisms causing nodules on ten wild legumes and on *Dolichos Lablab* were studied from the standpoint of their morphological, cultural, and cross-inoculation characteristics.

Cultural characterization of these organisms has been extended to include their fermentation of numerous carbohydrates, their reactions on amino acid media, and their sensitivity to salts and dye.

These organisms appear to fall into two groups.

Group 1 includes the organisms causing nodules on *Amphicarpa bracteata*, *Baptisia tinctoria*, *Cassia nititans*, *Crotalaria sagittalis*, *Desmodium paniculatum*, *Dolichos Lablab*, *Genista tinctoria*, *Lespedeza hirta*, and *Lespedeza frutescens*.

The morphological and cultural characteristics of the members of group 1 are essentially the same as those of the organism from cowpea and the organism (*Rhizobium japonicum*) from soybean.

On the basis of inoculation experiments, the organisms of group 1 are further characterized by their ability to form nodules, in so far as tested, on any other legume of the group, and by the ability of nearly all strains to form nodules on cowpeas, and of certain strains on soybeans. Cultures from *Desmodium paniculatum* appear to form nodules more readily, and the nodules are of a more beneficial type, on the latter.

Further evidence of the relationship of the organisms of group 1 to the cowpea and soybean organisms is the occurrence of cross-inoculation irregularities among strains of the latter. This suggests that the capacity to form nodules on any but the original host plant may be a physiological characteristic not found under certain conditions in all strains of these Rhizobia.

The strains of group 1, therefore, appear to be closely related to one another and also to the cowpea and soybean organisms both in cultural and cross-inoculation characteristics. Variations in their cross-inoculation capacities, however, prevent the giving of a species name to these organisms or the definite placing of their host plants in either the cowpea or soybean inoculation group, if the present bases for determining species and inoculation groups be observed.

Group 2 includes the organisms causing nodules on *Lathyrus latifolius* and *Lathyrus japonicus*.

The morphological and cultural characteristics of the organisms of group 2 are essentially the same as those of *Rhizobium leguminosarum* from *Pisum*, *Vicia*, and certain species of *Lathyrus*.

The organism from *L. latifolius* is further characterized by its ability to form nodules on *Vicia* and on other species of *Lathyrus* and hence should be included in the pea group as previously suggested.

The organism causing nodules on *Lathyrus japonicus* is characterized by its ability to form nodules on other species of *Lathyrus* and by its irregularity in forming nodules on *Pisum* and *Vicia*. This irregularity, similar to that found among cultures of group 1, prevents the placing of *Lathyrus japonicus* without reservations in the pea group, or the conclusion that under the present species rules, the organism is *Rhizobium leguminosarum*.

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# THE NITRIFICATION PROCESS AND PLANT NUTRITION<sup>1</sup>

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Nitrate is the most abundant anion, except bicarbonate, resulting from biological processes in good soils. Likewise, if it is assumed that the plant takes its nitrogen in the nitrate form, nitrate is needed in larger amounts by plants than is any other anion. Nitric acid is strong and has a vigorous solvent action upon soil minerals.

What restricted nitrification, what processes in the soil take its place? Some plants apparently succeed where there is little nitrification. What is the significance of the nitrification process in the nutrition and growth of higher plants? The following considerations attempt to answer these questions.

## THE SOLVENT ACTION OF NITRIFICATION

The significance of nitrate as a carrier of cations cannot be appreciated from the quantity found at any given moment. The nitrate level in the soil at any time under crops may be low even though the nitrate has served in liberating from the soil and carrying into the plant a large portion of the cations needed for growth.

The large amounts of nitrate that may be produced and the solvent effect of nitrification are shown by calculations from data on an uncropped soil studied by Hibbard (14). The data are presented in parts per million by Hibbard, but the writer has converted them to milliequivalents per 10,000 gm. soil (table 1).

The data in table 1 indicate that the nitrate in the lower concentrations accounts for only a small part of the soluble cations but, as the nitrate increases, soluble cations increase markedly, and the nitrate accounts for a larger portion of their total. In the low concentrations of nitrate the sulfate is of about equal importance. With increased biological action the nitrate becomes increasingly important as a solvent, compared with sulfate. Perhaps in the lower concentrations of nitrate in non-acid soils, as suggested by Burd (5), most of the cations are held in soluble form as bicarbonates. When nitrate increases, the solvent effect of carbonic acid becomes correspondingly less significant.

With nitrate present in large amounts, cations must be in solution in relatively large amounts, though the converse is not necessarily true. Other anions can, of course, hold cations in solution.

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## RELATION OF SOIL REACTION TO THE SOLVENT EFFECT OF NITRIFICATION

The problems of plant nutrition are not identical in acid and alkaline soils. Carbonic acid partially neutralizes black alkali by forming a bicarbonate.

Data by Conrad (8) indicate that carbonic acid has little solvent effect upon soils more acid than pH 6.0. The dissociation constant for carbonic acid (6) likewise indicates that an acidifying and therefore solvent effect cannot be appreciable until the soil is about neutral or somewhat alkaline in reaction. McGeorge and Breazeale (21) showed that an alkaline calcareous soil of pH 8.4 could be reduced in a water suspension to pH 6.4 by carbonic acid.

Saline soils carry a relative abundance of salts in solution, largely chlorides and sulfates. Acid soils are the result of excessive leaching and removal of solutes. The more easily dissolved nutrients have long since disappeared. Solutes useful to plants, as they are liberated from day to day, must be brought into available form largely by biological processes that produce solvents ca-

TABLE 1

*Water extract of Yolo silty clay loam soil untreated (1-5), showing important nutrients  
M.e. per 10,000 gm. soil*

TIME OF IN- CUBATION	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>	SUM ANIONS	K	Ca	Mg	SUM CATIONS	CATIONS ACCOUNTED FOR BY NO <sub>3</sub>
days									per cent
0	4.2	1.6	4.5	10.3	7.2	19.1	14.9	41.2	10
11	9.0	1.4	6.5	16.9	8.8	24.9	16.6	50.3	18
40	16.4	2.1	4.0	22.5	5.8	36.7	16.6	59.1	28
90	18.6	1.7	3.9	24.2	9.3	34.7	19.3	63.3	29
160	23.7	1.7	5.8	31.2	11.4	23.0	20.4	54.8	43
254	46.1	1.5	13.0	60.6	10.8	20.9	34.9	66.6	69
395	51.1	1.6	10.0	62.7	12.5	25.5	29.6	67.6	76

pable of attacking the somewhat inert mineral soil. Under such conditions strong acids, such as nitric, must be the principal active solvents.

When active nitrification starts in the spring, soil acidity increases. The increase may amount to as much as 1 pH during the season but usually it is less (13, 24, 25). Later in the season, vigorous growth of plants depletes the soil of its nitrates, and the acidity is reduced. After harvest, another period of active nitrification again increases acidity. Changes in soil reaction follow closely upon changes in the accumulation of nitrates.

The solvent effect of nitrification is observed in the removal of bases in the form of nitrates leached from the soil during the winter (15). The leaching losses through a 4-year period at the Virginia Agricultural Experiment Station are summarized in table 2. Soils were treated in different ways, and the calcium, magnesium, potassium, sulfate, and nitrate that were removed by drainage waters were estimated. Soil was exposed in tanks, and the drainage water was collected for analysis. For convenience of quantitative comparison, the

losses of the various nutrient ions have been converted to hydrogen equivalents per acre. The data indicate that the nitrate loss is sufficient to account for two-thirds to three-fourths the cations removed. The pH of the soil is not given, but the leachings were nearly neutral.

TABLE 2

*Summary of 4 years' leaching losses of Ca, Mg, K, SO<sub>4</sub>, and NO<sub>3</sub> converted to hydrogen equivalents per acre (15)*

SOIL TREATMENT	GREEN RYE MULCH	GREEN RYE TURNED	MATURE RYE MULCH	MATURE RYE TURNED	CONTROL
Cation loss (Ca + Mg + K).....	34.10	34.65	26.88	24.42	21.48
Anion loss (SO <sub>4</sub> + NO <sub>3</sub> ).....	29.61	28.71	21.59	19.78	17.29
NO <sub>3</sub> loss.....	25.69	25.43	17.09	16.56	14.13
Cation loss accounted for by NO <sub>3</sub> ..... per cent	75.3	73.4	63.6	67.7	65.7

TABLE 3

*Important nutrients—nitrate, sulfate, calcium, and potassium—water soluble (1:5) in the soil  
M.c. per 10,000 gm. soil*

SOIL TREATMENT	ANIONS†		SUM ANIONS	CATIONS		SUM CATIONS	DIFFERENCE CATIONS AND ANIONS	Ca + K ACCOUNTED FOR BY NO <sub>3</sub>
	NO <sub>3</sub>	SO <sub>4</sub>	NO <sub>3</sub> + SO <sub>4</sub>	Ca	K	Ca + K	Excess Ca + K over NO <sub>3</sub> + SO <sub>4</sub>	
								per cent
Meyer clay adobe:								
Check.....	17.38	7.04	24.42	26.04	4.95	30.99	+6.57	56
*Alfalfa 5 tons.....	26.19	5.49	31.68	42.00	7.18	49.18	+17.56	53
Manure 5 tons, dry weight.....	23.33	7.99	31.32	30.16	7.27	37.43	+6.11	62
Aiken silty clay loam:								
Check.....	14.67	2.14	16.81	10.10	2.70	12.80	-4.01	115
Alfalfa 5 tons.....	31.67	3.12	34.79	20.33	6.16	26.49	-8.30	119
Manure 5 tons, dry weight.....	22.38	4.03	26.41	13.50	7.69	21.19	-4.22	105

\* Rates of treatment are for 2,000,000 pounds of soil. Treatments were made on potted soils in the greenhouse.

† The term "ion," in this paper is used in a general way, without any distinction between the ionized or the non-ionized form.

The data in table 3 indicate how the reaction of the soil and the reserve supply of mineral nutrients may affect the solvent action of biological processes. The soils were treated with two nitrifiable materials, alfalfa and stable manure.

The Meyer soil is nearly neutral in reaction and well supplied with the important nutrient cations. The exchangeable calcium is 0.630 per cent, and the exchangeable potassium is 0.091 per cent. There is more water-soluble calcium



and potassium than is equivalent to the nitrate and sulfate, indicating an active solvent effect of some other acid, hydrochloric or carbonic, probably the latter (5).

The Aiken soil is acid (pH 5.6) and base deficient. The exchangeable calcium is 0.120 per cent, and the exchangeable potassium, 0.038 per cent. To bring bases into solution in this soil requires the vigorous action of strong acids, nitric and sulfuric.

The result is that in the Meyer soil there is an excess of calcium and potassium over nitrate and sulfate and that in the Aiken soil there is a deficiency of calcium and potassium compared to nitrate and sulfate. Thus the reaction of the soil and the supply of cations in the soil complex determine how effective is the solvent action of the acids of varying strengths.

Nitrate in the acid soil is sufficient to account for more than both the calcium and potassium that are in soluble form. In the neutral soil, which contains approximately the same amount of nitrate and sulfate but much more calcium and potassium, the nitrate is equivalent to about half the calcium and potassium in solution, and other solvents are of greater significance.

#### THE COMPOSITION OF PLANTS AS INDICATIVE OF THEIR NUTRITION

Plants absorb from the soil and its solution anions which include  $\text{NO}_3^-$ ,  $\text{SO}_4^{--}$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{--}$ ,  $\text{SiO}_3^-$ ,  $\text{HCO}_3^-$ , and possibly others. The cations are  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Fe}^{+++}$ , and others. The form in which absorption occurs is uncertain.

Table 4 shows four each of the more common anions and cations and their quantitative importance for a number of crops. The data are calculated from Forbes' analyses (12).

The table shows nitrogen in an amount more than equivalent to the sum of the four important cations in most cases. The nitrogen is generally considerably more than equivalent to the other three important anions. Omission of the silicate anion, which was not reported in the analyses, does not affect the main purpose of the discussion.

The presence of nutrient ions in the plant indicates nothing of how they may have been absorbed. In priority of liberation in the soil, greater importance attaches to the anions since they are, for the most part, liberated from organic matter by biological processes. Liberation of anions indirectly controls the liberation of cations.

Plants of high nitrogen content should be high in cations, if nitrate is an important carrier of cations. Parker and Truog (22) point out the relation between high nitrogen and high calcium, though they were studying the relationship from another point of view. A rather extensive study would be required to establish this, at present, assumed relationship.

A study of the table (an incomplete list) indicates that plants contain more anions than cations (nitrogen being considered as an anion), though there are not sufficient data for arriving at the absolute sum of either. The plant, of

course, maintains a balance of ions. The balance may be adjusted by conversion of nitrate to ammonia and by the synthesis of organic ions. If carbonic acid is included as a solvent and carrier of nutrients in soils of favorable reaction, a balance of anion and cation in plant intake is facilitated. Under some conditions, the hydrogen of the carbonic acid may correct a deficiency of cations.

Possibly nutrients can be absorbed as acids, nitric acid (1, 7) for example. Breazeale and McGeorge state that phosphate is best absorbed as the  $H_2PO_4$  anion (4). If some of the nitrogen is absorbed as ammonia, a cation is furnished to balance anions.

But there is another helpful explanation providing for adequate absorption and nutrition of the plant without upsetting the balance of anion and cation if

TABLE 4  
*Anions and cations of common crops*  
M.e. per 100 gm.

CROP	ANIONS				SUM ANIONS	SUM OF S, Cl, P	N PER CENT OF TOTAL ANIONS	CATIONS				SUM CAT- IONS	CATIONS AC- COUNTED FOR BY N AS AN ANION
	N	S	Cl	P				K	Na	Ca	Mg		
													<i>per cent</i>
Alfalfa hay.....	165.0	17.5	4.2	21.3	208.0	43.0	79	19.7	19.5	51.5	30.3	121.0	136
Bluegrass hay..	104.3	19.4	6.2	21.3	151.2	46.9	69	32.9	5.8	15.5	18.0	72.2	144
Clover hay.....	148.6	11.3	6.8	16.5	173.2	24.6	86	43.4	2.6	57.0	22.1	125.1	119
Millet hay.....	68.6	9.4	3.4	16.5	97.9	29.3	70	32.4	3.9	15.5	20.5	72.3	95
Cowpea hay....	254.3	20.0	4.2	24.3	305.8	51.5	83	19.9	28.2	90.5	80.4	219.0	116
Timothy hay...	60.7	9.4	5.1	10.7	85.9	25.2	71	14.3	13.9	9.0	8.2	45.4	137
Soybean hay...	195.7	14.4	2.3	20.4	232.8	37.1	84	40.6	5.8	61.5	50.8	198.7	98
Wheat straw....	20.0	9.4	5.6	3.9	38.9	18.9	51	20.4	9.5	10.5	4.9	45.3	44
Wheat grain....	117.9	12.5	2.1	35.9	168.4	50.5	70	13.3	1.2	2.5	10.7	27.7	426
Corn stover....	62.9	10.6	8.2	9.7	91.4	28.5	69	43.9	2.6	23.5	7.4	77.4	81
Corn grain.....	99.3	9.4	1.7	25.2	135.6	36.3	73	8.7	0.9	0.5	9.0	19.1	520
Cabbage.....	13.6	3.8	0.6	1.9	19.9	6.3	68	4.3	0.0	2.0	1.6	7.9	172

nitrogen is absorbed as the anion. Almost immediately in the plant, the nitrogen is changed to the cationic form of ammonia (11) and combined with fatty acids to form amino acids. The cation with which the nitrogen was associated in entering the plant is thereby released to unite with some other anion, probably the bicarbonate, a fatty acid, or a by-product of plant metabolism. Parker and Truog (22) believe that an important function of calcium is to neutralize acid by-products of metabolism, which might otherwise prove toxic to the plant.

When any solute, potassium nitrate, for example, enters the plant, the change of the nitrate anion to the cationic form of ammonia liberates the potassium. Some of the potassium or other cations so liberated may combine with carbonic acid (produced abundantly by cell respiration) to form a bicarbonate. The

cation thus combined may diffuse from the plant cell into the soil to serve again as a base for nitrification. The same cation may serve repeatedly to carry anions, and particularly the nitrate anion, into the plant. This provides for maintaining a balance between cations and anions though the latter are used in larger quantities for growth. Though absolute proof may be questioned, migration of nutrients from the plant into the soil has often been assumed.

Thom and Humfeld present data (26) which indicate that the soil immediately adjacent to plant roots is kept nearer neutrality than the soil farther way. The authors do not offer an explanation for the effect. If bases are capable of passing from plant roots into the soil, that would account for the reduced acidity in acid soils. The excretion of carbon dioxide by the roots and microorganisms would account for the reduced alkalinity in alkaline soils.

The foregoing postulation may account for the mobilization of nutrients for plants absorbing large quantities of nitrate. Is the situation different for legumes? Legumes usually absorb and assimilate large amounts of bases, especially calcium and potassium. The legume gets much of its nitrogen from the air through the organisms in its roots. This might result in a reduced absorption of nitrate from the soil and give rise to the problem of providing a solvent and carrier to mobilize the needed cations. But legumes absorb nitrate from the soil to the extent that it is available, and fix nitrogen only to meet additional needs.

In addition to the nitrogen supplied symbiotically, which probably averages nearly half the total nitrogen obtained, enough more would be needed from the soil in the form of nitrate to account for most of the cation needs. In addition, many legumes thrive only on soils well supplied with bases and nearly neutral or somewhat alkaline in reaction, a condition in which carbonic acid is more effective as a solvent. Plants can no doubt absorb bicarbonates.

#### ABSORPTION OF NUTRIENTS BY PLANTS IN ACID SOILS

As acidity and base deficiency in soils increase, the nutrition of most farm crops becomes correspondingly poor. In very acid soils nitrification may be greatly reduced and sometimes nearly absent. If nitric acid is an important nutrient and an effective solvent and carrier of cations, what can compensate for its lack when high acidity develops?

Ammonia, as shown by Allison (1, 2) and others (27), can be utilized by many plants but is poorly absorbed from acid solutions (27). Nitrate is more readily absorbed from an acid medium.

Ammonia does not dissolve mineral nutrients, and neither does it serve as a carrier of cations. In soils where more of the nitrogen is liberated in the form of ammonia, plants appear at a disadvantage in obtaining nutrients. Plants native to acid soils are low in cation content, as is indicated by a comparison of the mineral composition of conifers and hardwoods. The conifer growing in acid soil environment has adjusted itself to low mineral needs. The same relationship holds for pasture grasses grown on good and poor soils. As the soil

becomes acid and low in nitrifiable organic matter the better grasses give way to those of poor quality, low in both minerals and protein (9).

Joffe (18) presents ash analyses of litter (old leaves) from various sources for both conifers and hardwoods. After averaging several analyses he states: "The average ash content of conifers is 2.54 per cent, with a minimum of 1.29 per cent and a maximum of 3.31 per cent, whereas the average from the hardwoods is 6.63 per cent with a minimum of 3.85 per cent and a maximum of 11.74 per cent." The hardwood leaves average 2.6 times as high in ash as the conifers.

Plants probably are not entirely dependent upon solutes in the soil for nutrition. Jenny (17) has shown that the soybean is capable of obtaining calcium directly from the exchange complex of the soil colloids. Hydrogen was excreted by the plant root and exchanged for the calcium. Jenny found that the plant was at a disadvantage when compelled to take calcium from this source, after the calcium concentration in the colloid was reduced to a low level.

Nature makes some provision for the nutrition of plants which are native to acid, base-deficient, ammonia-producing soils. There is the possibility of a symbiotic relation between certain fungi and the host plant. The fungi, both endophytic and ectophytic, grow on the roots of the host, and the fungi-infected root, designated a mycorrhiza, is the absorbing organ of the plant. The mycorrhiza are abundant on acid plants, particularly conifers, heaths, and orchids. The natural habitat of these plants is in acid humus, or soils rich in acid humus, where little nitrate is produced.

The fungi have been found on the roots of many other plants, but on plants which grow in good nitrate producing soils only a pseudo-mycorrhizal development occurs. Jones (19) states that he found mycorrhizal roots on nearly all the common legumes wherever grown but that with favorable conditions rapidly growing roots soon outgrow the fungus. The fungus is present merely incidentally rather than as serving an important function.

That mycorrhiza are helpful to plants growing in acid soils has been both advocated and disputed by several workers (23). There are several points that are indicative of the helpfulness of the mycorrhiza. The young and actively absorbing roots develop the mycorrhiza. In some nursery soils forest trees fail because of lack of the proper fungi for the mycorrhiza. When the fungi are introduced and the mycorrhiza develop, the trees thrive (23).

Rayner (23) states that, "Conifers and, in all probability, other trees, can utilize ammonium compounds, and possibly to some slight extent, more complex organic compounds of nitrogen, but the last named are much more readily used by the root fungi. Hence, on acid humus soils, in which such compounds constitute the chief source of nitrogenous nutrient, plants provided with mycorrhiza are extraordinarily well equipped for competition with soil organisms." The organisms bringing about decomposition have first chance at the rather limited supply of available nitrogen of raw acid humus.

Rayner in the course of his discussion refers to "Stahl's theory of nutrition," as related to mycorrhiza. The mycorrhiza are a very efficient mechanism for absorbing soluble salts from the soil. Higher plants with abundant root systems and many root hairs are efficient absorbers. In mycorrhiza plants the fungus largely replaces the root hair as an absorbing organ. Rayner (23, p. 56) defines Stahl's theory of nutrition: "Namely, that the demand for available mineral salts in certain soils exceeds the supply, the resulting intensity of competition being the primary cause of a symbiotic relation in mycorrhiza." Thus the mycorrhiza are helpful to the plant not only in obtaining nitrogen, but in obtaining minerals also.

McArdle (20), in discussing the relation of mycorrhiza to conifers, states that the fungus may enable the trees to absorb water better, and may aid also in the absorption of mineral nutrients. Most of the mycorrhiza are found in the upper layers of soil where the tree gets its nutrients. He does not find any indication of injurious effects caused by the fungus of the mycorrhiza.

From the standpoint of energy economy, the absorption and utilization of ammonia should prove advantageous to plants. Nitrate is reduced to ammonia in the plant as the first step in protein synthesis. Results do not indicate an advantage, however, but rather a disadvantage. Lack of nitrate eliminates some species in a competitive existence. Others have become accommodated to an ammonia nutrition through symbiotic relation with fungi. But even these plants are probably better nourished in an environment where some nitrification occurs. In very acid soils there is often some nitrification.

Rayner again says (23, p. 216): "It is well to recall the fact that in the plant world, the severity of the struggle for existence not uncommonly centers about the competition for suitable compounds of nitrogen. . . . Even the rôle of vascular plants in general as 'nitrate organisms' may be so regarded, forming, as it does, an indispensable link in that remarkable cycle of chemical changes by which the 'circulation of nitrogen' is secured and maintained in nature."

#### GENERAL SIGNIFICANCE OF NITRIFICATION IN SOILS

There is good evidence that nitrification is of more than passive significance in plant nutrition. Nitrates are absorbed and renewed in the soil many times throughout the growing season. No other strong acid (in the form of its salt) is so abundant in any but saline or alkali soils. No other anion (if absorption of nitrogen in the anion form is assumed) is needed in such large amounts for plant growth.

With nitrification eliminated there are no adequate means for dissolving a sufficient quantity of the cations of the mineral soil (1) and for getting sufficient of either nitrogen or the cations into the plant (2). The mycorrhizal arrangement, while helpful to certain plants, can hardly be considered adequate. The mineral nutrition of crop plants on very acid soils is not satisfactory.

Building up the nitrifying power of poor sour soil through the addition of

high nitrogen organic matter and the use of lime to neutralize acids is favorable to the nutrition of common plants. Observation indicates that as soils become poor and base deficient, the renewal of organic matter which may support nitrification becomes increasingly important. Legumes may succeed with little organic matter if there is sufficient basic material to maintain the soil near neutrality.

Humid subsoils, usually somewhat acid, low in organic matter, and possessing little biological activity are characteristically raw, or refuse to give up mineral nutrients even to legumes which are presumably able to obtain sufficient nitrogen from the air. A bad physical condition is often assigned as the cause for the rawness. Perhaps it is not the only cause. Incorporation of organic matter and the stimulation of the nitrification process entirely change the subsoil character. Mineral nutrients are liberated and plant growth is supported. The subsoil of arid areas, by contrast, are usually not raw to legumes. These subsoils are alkaline in reaction and carry a good supply of easily soluble minerals. Carbonic acid given off by the roots of the plant is capable of bringing into solution and carrying into the plant some of the minerals so easily accessible.

Jenny (16) has correlated crop yields and land values on a broad basis with the nitrogen and organic content of soils. Although his data do not treat this phase, crop yields undoubtedly would correlate even more closely with the nitrifying power and the production of nitrates in the soil (28). Whenever conditions are favorable for complete mineralization of the organic matter, which means abundance of nitrate in soils that are rich in organic matter, there is also an abundance of available mineral nutrients important to plant growth. Among essential soil processes, therefore, nitrification stands high in its importance to the nutrition and growth of higher plants.

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# SULFUR REQUIREMENTS OF AZOTOBACTER CHROOCOCCUM

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All living organisms, including bacteria, require sulfur for normal growth and metabolism. Some forms of life require it in the organic form, whereas others require it in the inorganic form. To which class does *Azotobacter chroococcum* belong? Some soils are extremely low in sulfur; hence, it may occasionally be the limiting factor in crop production (5). If sulfates are added to some soils low in sulfur, nitrogen fixation is increased (4), and when small quantities of such a soil are incubated in a liquid medium a profuse chocolate-brown membrane appears. The growth is often scant in quantity and of a light color when sulfates are deficient (1). The increased bacterial activity occurring when sulfur and sulfur compounds are added to soil may be due to sulfur acting as a nutrient or to the liberation from the soil of other essential nutrients, for it is rather generally known that sulfates are powerful soil stimulants (3). The work reported in this paper deals with the nitrogen fixed in pure cultures of *Azotobacter chroococcum* grown in a basic liquid medium to which colloidal sulfur and various sulfur compounds were added in varying concentrations. This rules out the indirect effect which may occur in soils and answers the question: What forms of sulfur are used by *Azotobacter chroococcum*?

## METHODS

A strain of *Azotobacter chroococcum* isolated from the College Farm soil was used. It was cultured on a synthetic medium (2) and examined periodically to insure purity. The basic medium to which the colloidal sulfur and sulfur-carrying compounds were added had the following composition:

KH <sub>2</sub> PO <sub>4</sub> .....	0.02 per cent
MgCl <sub>2</sub> ·6H <sub>2</sub> O.....	0.02 per cent
NaCl.....	0.02 per cent
CaCl <sub>2</sub> ·H <sub>2</sub> O.....	0.01 per cent
FeCl <sub>3</sub> .....	50 p.p.m. iron
NaI.....	40 p.p.m. iodine
MnCO <sub>3</sub> .....	40 p.p.m. manganese
CaCO <sub>3</sub> .....	1.0 per cent
Mannitol.....	1.5 per cent
Distilled water.....	1,000 cc.

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<sup>2</sup> Contribution from department of chemistry and bacteriology. Publication authorized by director, September 3, 1935.



Baker's chemicals of highest purity were used. No attempt was made to free them from sulfur impurities; hence, the basic medium always carried a minute constant of sulfur. To this basic medium were added, both separately and in varying quantities, mustard oil, sodium thiocyanate, sodium thiocarbonate, sodium sulfocarbolate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sulfur iodide, sulfonmethane, sodium sulfanilate, sodium sulfide, sodium sulfite, cysteine, and ethyl mercaptan. One hundred cubic centimeter portions of the different media were distributed in 500-cc. Erlenmeyer flasks and sterilized in an autoclave. One-half of the flasks were inoculated with 1 cc. of a suspension of *Azotobacter chroococcum*; the other half were

TABLE 1

*Nitrogen fixed in the presence of varying forms and quantities of sulfur*

	NITROGEN FIXED IN MEDIA CONTAINING				
	No S	5 p.p.m. S	10 p.p.m. S	50 p.p.m. S	100 p.p.m. S
	mgm.	mgm.	mgm.	mgm.	mgm.
Mustard oil (added after autoclaving).....	1.2	1.4	1.3	0.9	0.6
Mustard oil (added before autoclaving).....	1.2	1.3	1.4	1.4	0.5
Sodium thiocyanate.....	1.2	1.3	1.2	1.4	1.3
Sodium thiocarbonate.....	1.2	4.1	4.3	4.4	4.4
Sodium sulfocarbolate.....	1.2	1.3	1.2	1.2	1.4
Sodium ethylsulfate.....	1.2	4.1	3.9	4.4	4.5
Colloidal sulfur.....	1.2	3.8	3.0	4.0	4.0
Sulfur chloride.....	1.2	4.4	4.9	3.9	4.2
Sulfuryl chloride.....	1.2	5.2	5.0	5.0	3.4
Sulfur iodide.....	1.2	.....	0.8	0.5	.....
Sulfonmethane.....	1.2	1.3	1.3	1.4	1.4
Sodium sulfanilate.....	1.2	1.4	1.2	0.7	0.1
Sodium sulfide.....	1.2	4.6	4.6	4.3	4.6
Sodium sulfite.....	1.2	4.0	4.2	4.1	4.3
Cysteine hydrogen chloride.....	1.2	4.1	3.8	3.9	3.2
Ethyl mercaptan (added after autoclaving).....	1.2	1.3	1.3	1.3	1.3
Ethyl mercaptan (added before autoclaving)....	1.2	1.5	1.6	1.7	1.9
Control media (2) (sulfate).....	1.0	.....	.....	4.8	.....

left sterile. All were incubated at 28°C. for 5 weeks; the nitrogen was determined by the Kjeldahl method, and the sulfate sulfur, by the Woodman and Evans method (6). The number of milligrams of nitrogen fixed in the presence of colloidal sulfur and various sulfur-carrying compounds are given in table 1. Each reported result is the average of three or more closely agreeing determinations.

Since ethyl mercaptan and mustard oil are volatile, they were added to some flasks before autoclaving and to others after autoclaving. The gain in nitrogen in the presence of the heated and non-heated ethyl mercaptan was low, indicating the unavailability of the sulfur.

When the mustard oil was added after autoclaving there was no growth at the higher concentrations and little or none at the lower concentrations, thus indicating that mustard oil is toxic. When mustard oil was added before autoclaving, considerable growth occurred but no fixation. In this case it appears that the mustard oil was hydrolyzed, the resulting compounds not being toxic; however, nitrogen fixation was discouraged because of the combined nitrogen. Traces of sulfur in the chemicals were no doubt sufficient for the small gains in nitrogen found in the checks (those receiving no sulfur). There was slight early growth in the checks, which ceased about the fourth or fifth day of incubation. No nitrogen was fixed in the presence of sodium thiocyanate or sodium sulfanilate. This was not due to toxicity, but the combined nitrogen discouraged fixation by *Azotobacter chroococcum*. This is shown by the profuse growth in the medium to which these compounds were added; when the nitrogen was determined the quantity found, however, was no greater than that added in the sulfur-carrying compound. Although the cysteine supplied combined nitrogen it did not interfere with nitrogen fixation. The gain in nitrogen was slightly less in the presence of 150 p.p.m. of cysteine over that in the presence of smaller quantities. No fixation occurred, nor was there appreciable growth, in the presence of sodium sulfocarbolate, sulfur iodide, and sulfonmethane. No growth appeared in the presence of sulfur iodide, apparently because of its toxicity; whereas scant growth occurred in the presence of sodium sulfocarbolate and sulfonmethane, apparently because of the unavailability of sulfur in these compounds.

Sodium thiocarbonate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sodium sulfide, sodium sulfite, and sodium sulfate are all valuable carriers of sulfur to *Azotobacter chroococcum*, judged from gains in nitrogen occurring in their presence. The efficiency of the different sulfur carriers varies slightly with the concentration. Apparently, 5 p.p.m. of sulfur in any of the carriers was sufficient to meet the needs of *Azotobacter chroococcum* under these conditions. None of the sulfur carriers were toxic at 100 p.p.m.

The sulfates present in the sterile and in the inoculated media at the close of the incubation period were determined, and average results are given in table 2.

No sulfate was found in the sterile or in the inoculated media to which had been added mustard oil, sodium sulfocarbolate, sulfur iodide, sulfonmethane, sodium sulfanilate, and ethyl mercaptan. There were no gains of nitrogen in the media containing these sulfur compounds, indicating that the absence of sulfates predicates the absence of nitrogen fixation by *Azotobacter chroococcum*. At the end of the incubation period the sterile media to which had been added sodium thiocyanate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sodium sulfide, sodium sulfite, and cysteine carried from 8 to 100 per cent of the added sulfur as sulfates. As nitrogen fixation progressed, the sulfates disappeared; they disappeared completely from the media containing sodium thiocyanate, colloidal sulfur, and cysteine hydrochloride. When

mustard oil was added before autoclaving, sulfates appeared; when added after autoclaving, no sulfates appeared. A comparison of results in tables 1 and 2 shows that in those media in which sulfates appeared there were gains in nitrogen, whereas in those in which sulfates did not appear there were no gains in nitrogen. An exception was sodium thiocyanate; here the combined nitrogen apparently discouraged fixation.

From these data it appears that *Azotobacter chroococcum* requires sulfur in the form of sulfates, and all compounds which are spontaneously oxidized to sulfates or compounds which are converted by bacterial activities into sulfates may serve as valuable sources of sulfur. The limited number of com-

TABLE 2

Percentages of original sulfur recovered as sulfates from incubated media to which had been added 50 p.p.m. of colloidal sulfur and different sulfur-carrying compounds

	PROPORTION OF ORIGINAL SULFUR RECOVERED AS SULFATES	
	Not inoculated	Inoculated
	per cent	per cent
Mustard oil.....	0	0
Sodium thiocyanate.....	12.9	0
Sodium thiocarbonate.....	76.4	47.8
Sodium sulfocarbonate.....	0	0
Sodium ethylsulfate.....	100.0	98.6
Colloidal sulfur.....	8.0	0
Sulfur chloride.....	52.5	26.4
Sulfuryl chloride.....	93.7	89.6
Sulfur iodide.....	0	0
Sulfonmethane.....	0	0
Sodium sulfanilate.....	0	0
Sodium sulfide.....	66.5	52.8
Sodium sulfite.....	100.8	96.2
Cysteine hydrochloride.....	35.5	0
Ethyl mercaptan.....	0	0

pounds tested points to the conclusion that *Azotobacter chroococcum* can neither oxidize sulfur and sulfur-carrying compounds to sulfates nor use other compounds of sulfur.

#### SUMMARY

*Azotobacter chroococcum* was cultured in a basic nutrient medium to which were added colloidal sulfur and various sulfur-carrying compounds in varying quantities. Because of the toxicity of the compound, *Azotobacter chroococcum* did not grow in the presence of mustard oil and sulfur iodide, even when 5 p.p.m. were added. No nitrogen was fixed in the presence of sodium sulfo-carbolate and sulfonmethane, because of the unavailability of sulfur, and none was fixed in the presence of autoclaved mustard oil, sodium thiocyanate, and

sodium sulfanilate, not because of toxicity, but because of the combined nitrogen content of the compounds, which discouraged nitrogen fixation. Cysteine supplied combined nitrogen, yet it did not discourage nitrogen fixation.

Sodium thiocarbonate, sodium ethyl sulfate, colloidal sulfur, sulfur chloride, sulfuryl chloride, sodium sulfide, sodium sulfite, and sodium sulfate all are valuable carriers of sulfur to *Azotobacter chroococcum*. Judged from the gains in nitrogen occurring in their presence, apparently *Azotobacter chroococcum* requires sulfur in the form of sulfates and all compounds which are spontaneously oxidized to sulfates or compounds which are converted by bacterial activities into sulfates may meet the sulfur requirements of *Azotobacter chroococcum*.

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# THE EFFECT OF FERTILIZERS AND LIMING UPON THE ELECTRODIALYZABLE MANGANESE OF SASSAFRAS SILT LOAM<sup>1</sup>

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Since the soil is a dynamic system in which the equilibrium is constantly undergoing changes brought about by such factors as hydrogen-ion concentration, moisture, temperature, bacterial action, removal of ions by plants and their addition by fertilizers, the effects of subjecting a soil to a definite program of fertilizers and cropping over a long period should be reflected in the nature and amount of ions adsorbed by the soil.

During the course of an investigation of the effect of fertilizers, liming, and cropping on the electrodialyzable bases of Sassafras silt loam, the results obtained for manganese appeared to be of sufficient interest to warrant their publication prior to completion of the investigation.

## REVIEW OF LITERATURE

Schollenberger and Dreibelbis (14) while investigating the effect of cropping with various fertilizers, manure, and lime treatments upon the exchangeable bases of plot soils found no consistent indications of any very marked influence on the exchangeable manganese by fertilizers. The manure plots were notably high in exchangeable manganese; the most acid of the fertilizer plots, however, was the highest.

Davidson (3) employed water cultures to determine the effect of different ions on the absorption of manganese by wheat seedlings and found that the availability of manganese was governed by the combined effect of hydrogen-ion concentration and the anions present. Absorption was inhibited by phosphates but was greater with chlorides and sulfates than with the oxides.

Mann (7) found that manganese absorbed by plants exhibited a curve which closely followed that of the solubility of this element in the soils.

Willis (16, 17, 18) cautions against the use of the absorption by plants as an index of availability of manganese on account of the physiological interference of calcium compounds and stresses the importance of placing the factor "oxidation-reduction potential" on a quantitative basis.

Smolik (15) concluded that all soil manganese is reduced to manganese capable of exchange with simultaneous increase in pH of the soil.

Manganese deficiency, which is often induced by liming, may be corrected by the addition

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of soluble manganese salts (6, 17), manure (5, 13, 14), or physiologically acid fertilizers (1, 4, 6, 8, 9).

#### EXPERIMENTAL

##### *Treatment of plots*

The plots from which the samples were taken for the investigation are designated as "Block B" of the soil plots at the experiment station farm. The soil is classified as Sassafras silt loam.

The treatments to which the plots were subjected over a period of 24 years are indicated in table 1. Each plot (1/10 acre) was divided into two sections, one of which received lime as follows: 1908, 1916 at the rate of 2000 pounds per acre; 1912, 1920, 1924 at the rate of 1500 pounds per acre. The last

TABLE 1  
*Treatment of plots, 1908-1932*  
Total in pounds per acre

PLOT NUMBER	MURIATE OF POTASH	SODIUM NITRATE	DRIED BLOOD	SUPER PHOSPHATE	FARM MANURE
					<i>tons</i>
4	1,335				
6		Check			
7	1,335			3,900	
8	1,335	1,675			
9	1,335	1,599	75	3,900	
10	2,550	3,150	100	7,300	
11		Check			
14				750	65
15					130
16		Check			

application of lime was made in 1924. Both the limed and unlimed sections received the same fertilizer and manure treatments.

The following cropping system was employed on the plots from 1908-1912: corn, oats, wheat, and timothy + clover; from 1912, it has been corn, soybeans, wheat, and timothy + clover.

##### *Sampling*

Samples were taken in the late summer of 1932. Twenty cores ( $1\frac{5}{8}$  inch x  $6\frac{3}{4}$  inch) were taken from each section (limed and unlimed) of each plot with the utmost care to insure samples as representative as possible. Each sample was thoroughly mixed, air-dried, and again quartered.

##### *Electrodialysis*

One hundred grams of the air-dried soil was electrodialyzed in a three compartment Mattson cell<sup>3</sup> using parchment membranes and perforated platinum

<sup>3</sup> As listed by Arthur H. Thomas Company, Philadelphia, Pennsylvania.

sheet electrodes. With this arrangement the electrodes were approximately 2 cm. apart. Current was applied from a motor generator at 110 volts. The soil was subjected to electro dialysis for 1 hour, the dialyzates were removed, and electro dialysis was continued for three 8-hour periods, making a total of 25 hours for each sample.

TABLE 2

*Effect of fertilizers and liming upon total and electro dialyzable manganese of Sassafras silt loam*  
M.E. manganese (as Mn) per 100 gm. dry soil\*

PLOT	TREATMENT	TOTAL	DIALYZED	PER CENT DIALYZED	pH
4 { L NL	K	3.06 2.47	0.582 0.164	19.02 6.64	6.72 5.28
6 { L NL	Check	2.44 3.28	0.618 0.334	25.33 10.18	6.92 5.43
7 { L NL	PK	2.44 3.28	0.674 0.503	27.62 15.33	6.87 5.51
8 { L NL	NK	2.39 3.53	0.528 0.255	22.09 7.23	6.71 5.68
9 { L NL	NPK	2.55 3.53	0.429 0.205	16.82 5.80	6.85 5.56
10 { L NL	2(NPK)	2.47 3.31	0.703 0.559	28.46 16.89	6.63 5.59
11 { L NL	Check	2.91 2.88	0.706 0.370	24.26 12.84	6.93 5.42
14 { L NL	P + Manure	3.57 2.95	0.900 0.646	25.21 21.89	6.80 5.47
15 { L NL	2 Manure	3.57 3.24	0.760 0.550	21.28 17.00	6.64 5.53
16 { L NL	Check	2.94 3.20	0.615 0.529	20.92 16.53	6.70 5.44

\* 18 hours at 110°C.

L, limed. NL, Not limed.

### Results

The course of the current was remarkably uniform throughout the series, rising to a maximum of approximately 300 milliamps within a few minutes and decreasing gradually as dialysis progressed until it became practically constant at 30 milliamps. Analyses of the dialyzates are presented in table 2.



The pH measurements were made after the soils had stood with occasional shaking for 3 days in 2.5 times their weight of water. The glass electrode (MacInnes-Dole type) with electron-tube potentiometer was employed.

As indicated in table 2, electro dialysis removed a greater amount of manganese from the limed plots without exception. The fertilizer plots and the checks (except 16) show the greatest percentage variation between the limed and the unlimed sections. The percentage yield for manganese was lowest in plots 4 and 9 (limed), and 4, 8, and 9 (K, NK, and NPK respectively) of the unlimed plots. The manure plots were high in dialyzable manganese.

With the exception of plots 4 (KCl), 11 and 16 (checks), 14 (P+manure), and 15(manure), the limed plots contained less total manganese than the unlimed plots.

Although the pH of plot 4 (KCl only) is the lowest, there is no marked variation within each group brought about by the treatments. The effect of liming on the reaction of the soils is apparent.

#### DISCUSSION

If the amount of manganese removed by electro dialysis is assumed to be an index of the availability of this element to plants it will be observed that the foregoing results are in conflict with the conclusions drawn from the results obtained by numerous investigators, i.e., that the availability of manganese decreases with decreasing hydrogen-ion concentration and is reduced to a low level by liming, for without exception the plots receiving lime, and consequently with the lower hydrogen-ion concentration, yielded more manganese than did the unlimed plots.

Since it has been shown (11) that electro dialysis and neutral salt solutions extract essentially equivalent amounts of bases from soil colloids (and it is reasonable to assume that the action on whole soils would be comparable) and since Schollenberger and Dreibelbis (14) employed neutral salt solutions and found that the manure plots were notably high but that the most acid of the fertilizer plots was the highest in exchangeable manganese, it is logical to assume that the amount of manganese removed by electro dialysis could be employed as an index of availability of this element in the plots under consideration. Analyses of samples of mixed hay from plots 10 and 11 showed that hay from the limed sections contained very little manganese whereas that from the unlimed sections contained appreciable amounts. The foregoing assumption is therefore unjustified.

The most obvious explanation of the results reported would be that as electro dialysis progressed the pH of the system was lowered with resulting increase in availability of manganese. If liming decreases the availability then a conservation of manganese should be reflected in the total manganese of the limed plots, yet the analyses indicate that in several of the limed sections manganese is lower than in the corresponding unlimed sections. Undoubtedly some factor or factors other than reaction alone influence the availability of

manganese, for it has been found that in some cases manganese is readily available at hydrogen-ion concentrations well above neutrality.<sup>4</sup> It was also found that chlorosis, which occurred during the summer growing season and which was corrected by the addition of manganese sulfate, did not occur on the same plots during cool, wet weather (6, 19).

These results and those of Mann (10) and Willis (16, 17, 18) suggest the possibility of an enhancement of the reduction of manganese by water-logging of limed soils.

Shortly after the plots were laid out it was found necessary to drain a section of the blocks. The tile crosses plots 15 and 16 diagonally. In 1928, lateral drains were laid through both sections (limed and unlimed) of each block.

Analyses of mixed hay from these plots indicate that the plot soils react normally as regards manganese during the hot, comparatively dry season. Therefore, the loss of manganese from the limed sections (which cannot be attributed to increased yield alone) must be caused by greater leaching from the limed than from the unlimed soil during the cold, wet months.

Although the plots were not laid out with studies of this character in mind, they furnish evidence of the effects of variations in physical condition of the soil upon its chemical character. A check on the manganese status of these plots some years hence should reflect the effect of draining the remainder of the plots.

Unfortunately, samples of these soils which were taken when the plots were established have been lost, and analyses which would be of value in establishing the original status of manganese are not available.

#### SUMMARY

Samples from a series of soil plots which had been subjected to a definite schedule of treatments during a period of 24 years were electrodyalyzed in order to determine the effect of these treatments upon the amount of manganese removed by this method.

Without exception, the limed soils yielded the greater amount of manganese. On a percentage basis plots 4 and 9 of the limed group, and plots 4, 8, and 9 (K, NK, and NPK respectively) of the unlimed group yielded the least.

Analyses of mixed hay indicated that very small amounts of manganese were removed from the limed plots, whereas appreciable amounts were removed from the unlimed, yet certain limed plots contained less total manganese than did the corresponding unlimed plots.

The results indicate the possibility of greater leaching from the limed plots during the cold, wet months, which may be attributed to the enhancement of reducing conditions brought about by water-logging of limed soils.

Although the KCl unlimed plot had the lowest pH, there was no marked variation within each group resulting from the treatments.

<sup>4</sup>From unpublished data of Dr. T. G. Phillips, N. H. Agricultural Experiment Station, Durham, New Hampshire.

No consistent indications of any marked influence of fertilizers on the electrodialyzable manganese of the plot soils were observed.

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## SORPTION IN AN IDEAL SOIL

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The ideal soil is an assemblage of spheres packed at random; it possesses a structure which, if the spheres are not too large, renders it an absorbent. It may accommodate masses of liquid condensed from the vapor state into capillary bodies; i.e., capillary condensation. It may, depending on the grain size, also offer an internal surface sufficiently large to hold an appreciable amount of liquid and render it an adsorbent as well; the molecules of the sorbed vapor are held in the form of an adsorbed layer, generally monomolecular, which extends over the available surface of the grains. Thus if into a quantity of soil, initially dry, vapor is introduced at a pressure below that required for saturation, a certain fraction of the molecules will condense into capillary masses of liquid enmeshed in the packing and the balance will be adsorbed on the parts of the total grain surface not covered by these masses. We shall first consider the capillary condensation of vapor in the soil.

The capillary surfaces possible to the condensed liquid masses are, in many respects, similar to those existing after drainage of an initially saturated soil when equilibrium has been established. In either case the physical processes involved are subject to the laws of thermodynamic equilibrium between a liquid and its vapor when separated by a capillary interface. Elsewhere (8) it has been shown that these capillary bodies may be simple rings of liquid around contact points, webs of liquid extending over two or more contacts, surfaces confining small masses of liquid which saturate the pore space of several neighboring grains, or, finally, the extended capillary surface present when saturation exists as in the case of capillary rise. In a column of soil sufficiently short, so that gravity effects are negligible, only one of these surface types will be found; if, however, the column is long, then part or all, depending upon its length and the maximum vapor pressure prevailing, may exist. The behavior may be better understood by following the hydration of a small sample of an initially dry soil.

We suppose a sample of soil sufficiently small that the liquid distribution is homogeneous and not affected by gravity, and follow the capillary condensation from zero vapor pressure to that prevailing over a plane surface. We suppose, first, that the vapor is introduced at very small vapor pressure. The

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condensed vapor must accumulate at the grain contact points and form rings of liquid wrapped around the axis of each pair of grains; otherwise the energy of the system would not be a minimum. If the vapor pressure is increased to a new value, more vapor must be supplied to maintain equilibrium; the additional vapor will condense on the rings already formed and increase their size. As the vapor pressure is further increased, the process of growth will continue until the rings overlap and merge. The boundary is then no longer capable of sustaining, everywhere, a distribution of single rings. A second type of capillary mass begins to exist, a web of liquid involving two or more contacts (the packing is not a regular array) and formed by the coalescence of several rings. These transformations are illustrated in figure 1. As the vapor pressure is still further increased, these masses likewise grow until they combine; the whole pore space becomes completely filled with liquid. There are, thus, for capillary condensation in the soil, three distinct stages of liquid distribution in the hydration process, each corresponding to a definite range of vapor

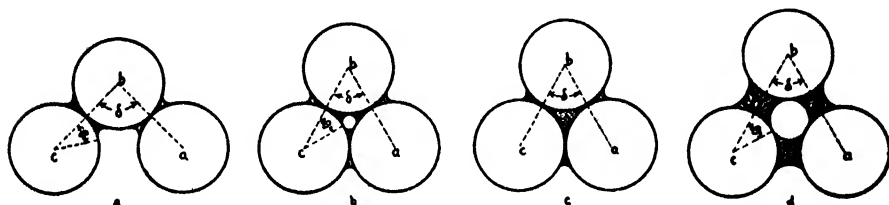


FIG. 1. TRANSFORMATION OF SIMPLE LIQUID RINGS

(a) Single rings with angle  $\delta > 60^\circ$ ; (b) Single rings about to merge in three grain pore with  $\delta = 60^\circ$ ; (c) Simple web of liquid existing after coalescence of rings; (d) Rings of liquid in spaced hexagonal packing, with  $\delta = 60^\circ$ .

pressures; at low vapor pressures the distribution consists of simple rings—the pendular stage (8). The succeeding or funicular stage is a stage of coalescence of the simpler forms and is characterized by the presence of webs of liquid. There is, finally, at vapor pressures near that over a plane surface, a stage of complete saturation.

The bounding surfaces of the condensed liquid masses are capillary and when at equilibrium possess definite curvatures which can be determined from the vapor pressures by the Kelvin equation (10). We shall use the following integral form of this equation:<sup>1</sup>

$$\int_S dS \cos \phi [(p - p_0) - (\rho p_0 / D_0) \log.(p/p_0)] = \sigma \int_s \cos \theta ds \quad (A)$$

The integrals extend, respectively, over the capillary surface  $S$  and around its bounding contour  $s$ .  $\phi$  is the angle between the normal to the surface-element  $dS$  and the vertical;  $ds$  is an element of the meniscus-solid contour;

<sup>1</sup> See Appendix 1.

and  $\theta$  is the angle between the vertical and that normal to  $ds$  which is tangent to the meniscus-surface.  $p_0$  is the vapor pressure just over the free liquid surface, and  $p$  is the vapor pressure over the element  $dS$ .  $\rho$  and  $D_0$  are, respectively, the densities of the liquid and vapor phases, and  $\sigma$  is the surface tension of the liquid.

It is permissible, for most purposes in soil physics, to neglect the differential variation in vapor pressure and to consider the vapor pressure constant over the meniscus; this is because such variations are due to gravity and are not in excess of the disturbance which would arise from a distance of one grain radius. With constant surface tension  $\sigma$ , we find, if  $a$  is the area of projection of the meniscus-surface on the horizontal, that

$$(p - p_0) - (\rho p_0 / D_0) \log_e(p/p_0) = \left( \sigma \int_s \cos \theta \, ds \right) / a \quad (B)$$

There are two additional approximations<sup>1</sup>

$$\frac{(\rho - D_0)}{D_0} (p - p_0) = \left( \sigma \int_s \cos \theta \, ds \right) / a \quad (C)$$

and

$$-(\rho p_0 / D_0) \log_e(p/p_0) = \left( \sigma \int_s \cos \theta \, ds \right) / a \quad (D)$$

Equation (C) is applicable only for vapor pressures near that of a plane surface. Equation (D) corrects for the compressibility of the vapor and is usually sufficient for most practical purposes; it enables us to find the vapor pressure over a capillary surface in equilibrium when the liquid-solid contour is known. We have then

$$p = p_0 e^{-\left( \sigma D_0 / \rho p_0 \right) \left( \int_s \cos \theta \, ds \right) / a} \quad (E)$$

Complete wetting will be assumed, and equations (B, C, D and E) will be applied to a uniform ideal soil, that is, one composed of identical spherical grains packed at random. The mass of the vapor absorbed under any given vapor pressure  $p$  and held as liquid by capillary condensation will, where possible, be estimated.

When vapor is first introduced into a dry soil, the distribution is pendular, and the capillary-condensed liquid is in rings around the grain contacts. The approximate volume of a single ring is easily found and is given by<sup>2</sup>

$$(v/2\pi r^3) = (\sec \theta - 1)^2 [1 - (\pi/2 - \theta) \tan \theta] \quad (F)$$

<sup>1</sup> Compare (8). Equation (F) was first obtained by Haines and Fisher; see also the derivation and references given by E. Manegold (6).

where  $r$  is the grain radius.  $\theta$  is the size of the ring—cf. fig. 2a—and is defined by the angle  $\theta$  included between the line of centers of the two grains support-

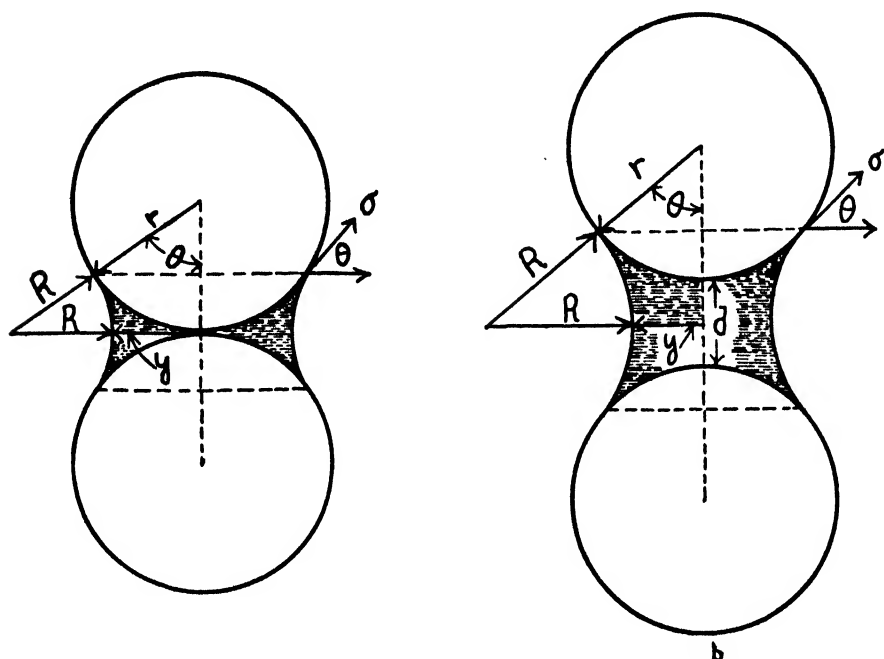


FIG. 2. (a) SIMPLE PENDULAR RING OF LIQUID. (b) SIMPLE RING OF LIQUID IN SPACED HEXAGONAL PACKING

ing the ring and the radius vector from the center of one of the grains to the outer edge of the ring on that grain (8). Setting

$$\lambda = -(\rho p_0 / \sigma D_0) \log_e(p/p_0) \quad (G)$$

we find on applying equation (D) to a simple ring that

$$\lambda = \frac{2(2\pi r \sin \theta) \cos \theta}{(2r - 2r \cos \theta) 2\pi r \sin \theta}$$

from which

$$\sec \theta = 1 + \frac{1}{r\lambda} \quad (H)$$

We find from equation (F) and (H)

$$v/(2\pi r^3) = (1/r\lambda)^2 \{1 - [\sin^{-1} 1/(1+1/r\lambda)] [(1/r\lambda) (2 + 1/r\lambda)]^{\frac{1}{2}}\} \quad (I)$$

as the volume of liquid held by two grains of radius  $r$  at their common contact in equilibrium under a vapor pressure  $p$ . The value of  $\lambda$  is obtained from

equation (G) from the known values of  $\rho$ ,  $\sigma$ ,  $D_0$ ,  $p$ , and  $p_0$ . A more accurate expression is obtained by use of equation (B). We set

$$\lambda' = (p - p_0) - (\rho p_0 / \sigma D_0) \log_e (p / p_0) \quad (J)$$

and obtain

$$\sec \theta = 1 + \frac{1}{r\lambda'} \quad (K)$$

An equation for  $(v/2\pi r^3)$ , identical with equation (J), is obtained from equations (F) and (K), except that  $\lambda$  is replaced by  $\lambda'$ .

To find the total quantity of liquid in the pendular region, we must know the total number of such rings in each cubic centimeter of packing. The number of contacts (two grains per contact) per cubic centimeter of packed space is equal to the number of rings existing in the same region and has been shown, elsewhere (8), to be given by

$$N = (3/(8r^3)) (1 + 1.828 x) \quad (L)$$

where  $x = (0.476 - P)/0.217$ ,  $P$  being the porosity of the packing. The total volume of retained liquid  $\Omega_p$  in 1 cc. of packed space is then

$$\Omega_p = Nv \quad (M)$$

where  $N$  is found by equation (L), and  $v$ , by equation (I). The total quantity of liquid held in a volume  $V$  is  $(\Omega_p V)$ . The relative retention is defined as the ratio of the liquid actually retained to that required to saturate the pore space; it is expressed, in the pendular region, by

$$\Omega_p/P = Nv/P \quad (N)$$

The mass of the absorbed vapor is easily found from equation (M) provided the densities of the liquid and its vapor, at pressure  $p$ , are known.

The pendular region is characterized by the presence of a ring distribution throughout; its bounding vapor pressures can be easily established. The ring size is governed by the vapor pressure [equation (H)], and all rings from zero radius up to the radius prevailing just before the first coalescence are permissible. The value  $p = 0$  defines the lower limit of the zone and corresponds to the ring of zero radius. Coalescence first appears in the smallest configuration of the packing—three grains, in contact, whose centers form an equilateral triangle (8). The angle between the axes of two adjacent rings in this configuration is  $60^\circ$ ; hence the critical value of  $\theta$  is  $30^\circ$ . Therefore, from equation (H), the rings first merge, during hydration, when

$$r\lambda = 6.464 \quad (O)$$



The critical vapor pressure  $p_c$  is obtained by solving equation (G) with  $\lambda = 6.464/r$ ;

$$p_c = p_0 e^{-\frac{\sigma D_0(6.464)}{r p_0}} \quad (P)$$

The same result can be obtained from equation (E) with  $\theta = 30^\circ$ . Equation (P) thus fixes the vapor pressure defining the junction of the funicular and pendular regions. All values of  $p$  such that  $0 < p < p_c$  will, when the packing is hydrated, result in a ring distribution throughout.

The funicular region is the zone of coalescence; coalescence not only of single rings but of more complicated forms. It commences when the pendular rings begin to merge and ends when saturation first appears. Conditions are more complicated, and several types of liquid configuration may exist. There may be single rings, for there are, in the ideal soil, many pairs of adjacent grains whose axes make an angle greater than  $60^\circ$  (fig. 1a);  $\theta$  may be greater than  $30^\circ$ , and rings larger than those in the pendular region may exist. There may also exist single webs of liquid formed by the coalescence of two or more rings; for example, the web existing in the pore cell of three adjacent grains, in contact, whose centers form an equilateral triangle, and formed by the coalescence of the rings originally existing at its contacts (fig. 1c). And, finally, there may be single cell-configurations completely saturated, because of the mergence of several neighboring rings or webs of liquid; for example, the regular tetrahedron, formed by the centers of four neighboring grains, will be completely filled when the rings associated with it merge.

An exact solution of the problem, in the funicular region, is difficult, and depends on a rigorous analysis of the packing problem. We shall only attempt to calculate a lower limit for the amount of liquid which can be retained when the funicular region is hydrated. As an approximation we shall resort to the statistical scheme of packing in which the grains are arranged in regular hexagonal array and spaced at a distance  $(2r + d)$  where  $d$  is a spacing constant adjusted to suit the porosity (8). We shall regard the liquid as distributed in the form of rings between each pair of grains of the stretched packing. This distribution represents the minimum possible in the hexagonal soil. The volume of the pore space occupied by the rings is certainly less than that taken up by the complicated masses present in an actual soil of the same porosity.

The size of the funicular ring is defined as before (fig. 2b). The angle  $\theta$  of the new ring is found, from equation (D), to be given by

$$q \sec \theta = 1 + \frac{1}{r\lambda} \quad (Q)$$

where  $\lambda$  is defined by equation (G) and  $q$  is defined by the equation  $q = (2r + d)/2r$ .  $q$  is found to be given in terms of the porosity by the equation (8)

$$q = 0.9045/(1 - P)^{1/3} \quad (R)$$

The volume of the ring is given by the expression (8)

$$v/(2\pi r^3) = q(q \sec \theta - 1)^2 [1 - (\pi/2 - \theta) \tan \theta] - (1/3)(q - 1)^2(q + 2) \quad (S)$$

From equations (Q) and (S) we find

$$v/(2\pi r^3) = (1/r\lambda)^2 \{q - [\sin^{-1} q/(1 + 1/r\lambda)] [(1 + 1/r\lambda)^2 - q^2]^{1/2}\} - (1/3)(q - 1)^2(q + 2) \quad (T)$$

Equation (J) may give a more accurate value; the resulting equations are identical with equations (Q) and (T) except that  $\lambda$  is replaced by  $\lambda'$  throughout. Equation (T) determines the volume of a "funicular" liquid ring in equilibrium under the vapor pressure  $p$ .  $\lambda$  is determined from equation (G) from the known values of  $\rho$ ,  $\sigma$ ,  $D_0$ ,  $p$ , and  $p_0$ . The total number of "funicular" rings per cubic-centimeter of packed space is given, as in an earlier paper (6), by

$$N_f = 9(1 - P)/(2\pi r^3) \quad (U)$$

The total volume of liquid  $\Omega_f$  retained in 1 cc. of packed space is therefore

$$\Omega_f = N_f v \quad (V)$$

where  $v$  is given by equation (T), and  $N_f$ , by equation (U); the value of  $q$  is found from equation (R). The retention in a volume  $V$  of packing is  $(\Omega_f V)$ . The relative retention is

$$\Omega_f/P = N_f v/P \quad (W)$$

The funicular masses, as the vapor pressure is increased, will grow until they coalesce and the packing becomes saturated. The funicular rings of the spaced hexagonal packing will merge when  $\theta = 30^\circ$  (Fig. 1d), and since the packing is symmetrical all must coalesce simultaneously, with resultant saturating of the packing. The small distortions required to transform the hexagonal soil, with grains separated by a distance  $(2r + d)$ , into an ideal soil, with random piling, will not alter this behavior to any degree; hence during hydration, both packings, if their porosities are equal, become saturated at the same pressure. We can, therefore, determine the vapor pressure  $p_*$  at which complete saturation occurs when the soil is hydrated; with  $\theta = 30^\circ$  we find from equations (Q) and (R) that

$$\frac{1}{r\lambda} = \frac{1.044}{(1 - P)^{1/3}} - 1 \quad (X)$$

and the critical pressure, that at which complete saturation first occurs during hydration, is given by

$$p_* = p_0 e^{-\frac{\sigma D_0}{r p p_0} \{1/[1.044/(1 - P)^{1/3} - 1]\}} \quad (Y)$$

All values of  $p$  such that  $p_c < p < p_s$  lie in the funicular region;  $p_c$  and  $p_s$  are the limiting vapor pressures.

The quantity of liquid held per cubic centimeter of packing in the saturation region is approximately equal to  $P$ , the porosity, and the total in a volume  $V$  of packing is  $(VP)$ .

After the packing becomes saturated, and the prevailing vapor pressure  $p$  becomes greater than the saturation pressure  $p_s$ , the only possible capillary

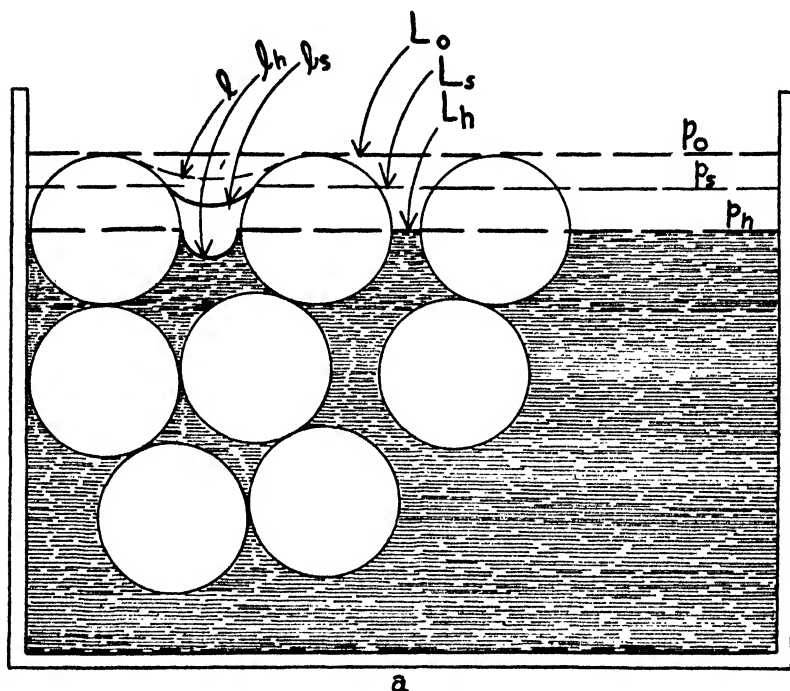


FIG. 3. POSSIBLE POSITIONS, IN THE BOUNDING GRAIN LAYER, OF A CONTINUOUS MENISCUS ENCLOSING A SATURATED PACKING

$l_h$  is a section of the meniscus between two grains when it occupies the plane of centers,  $L_h$ , at the vapor pressure  $p_h$ ;  $l_s$ ,  $L_s$ , and  $p_s$  are similar quantities for the minimum rise position;  $L_o$  and  $p_o$  are for a plane surface;  $l$  marks a section of a meniscus intermediate to  $L_o$  and  $L_h$ ; (a) packing with plane boundary; (b) packing with spherical boundary.

surface is that extending over the outer layer of grains bounding the packing; the packing remains saturated except in the bounding grain layer. If, for example, the soil is contained in an open cup, the capillary surface extends across the top layer of grains as in figure 3a. Any further increase in vapor pressure results in condensation on this outer boundary until the Kelvin relation is satisfied.  $L_s$ , in figure 3a, is the plane of the saturation meniscus when  $p = p_s$ ;  $L_o$  is the surface commonly tangent to the spherical grains and corresponds to the vapor pressure  $p = p_o$ , that over a plane surface of the liquid;

the maximum volume of additional liquid that can be condensed is closely that held between  $L_s$  and  $L_0$ . The outer grain layer can accommodate all curvatures between that of minimum capillary rise, corresponding to  $p_s$ , and zero, that of a plane surface for  $p_0$ ; the vapor pressure may, therefore, vary from  $p_s$  to  $p_0$ , while the amount of liquid condensed does not exceed the volume occupied by the grain layer on the outer boundary, and is, in the ordinary packed sample, a very small amount. Figure 3b shows the corresponding relations in

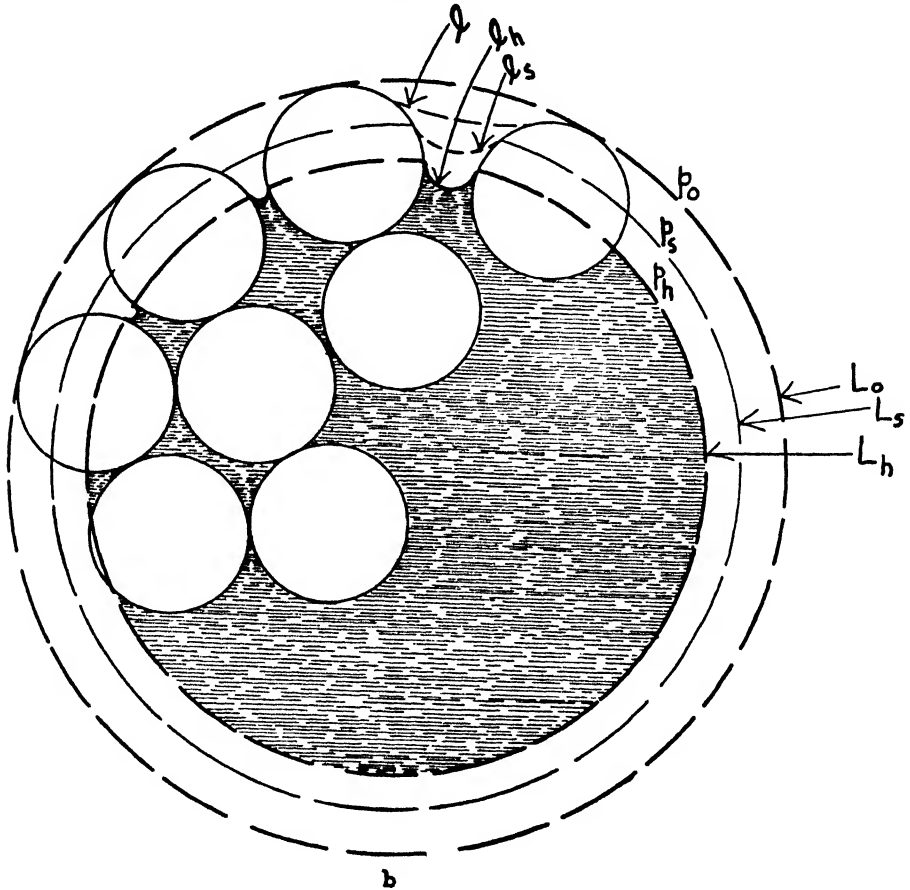


FIG. b

an isolated sample of soil with spherical boundary; the circles  $L_s$  and  $L_0$  enclose the region in which condensation may occur when  $p > p_s$ . It must be recalled that the samples represented in figures 3a and 3b are small, and condensation, since the vapor pressure is approximately constant throughout, occurs uniformly through the whole sample; a saturation meniscus must then lie in the outer boundary.

Figure 4a shows the relation between the relative retention and the vapor pressure  $p$ . The curve  $0_0 0_c 0_2 0_3$  is that of hydration.  $0_0 0_c$  defines the pendu-

lar region [computed from equation (N)];  $O_cO_f$ , the funicular, and is a limiting curve [computed from equation (W)]. The straight portion  $O_fO_s$  marks the passage from the funicular to the saturation region, under the constant vapor pressure  $p_s$  [computed from equation (Y)]; it is, likewise, a limiting curve. The hydration curve for an actual soil will lie beneath  $O_fO_s$ ; the curve corresponding to  $O_0O_s$  will lie within the "loop" of figure 4b. Complete saturation first occurs at  $O_s$  (neglecting the error introduced by the bounding grain layer).  $O_sO_2$  corresponds to the saturation region, just discussed, where  $p_s < p < p_0$ .

If, now, the packing is dehydrated, the hydration curve, for the most part, is not retraced. We shall follow the process. We suppose the soil sample initially saturated under a vapor pressure  $p_0$ ; the meniscus is then in the extreme edge of the outer grain layer,  $L_0$  of figures 3a and 3b. The outer boundary is

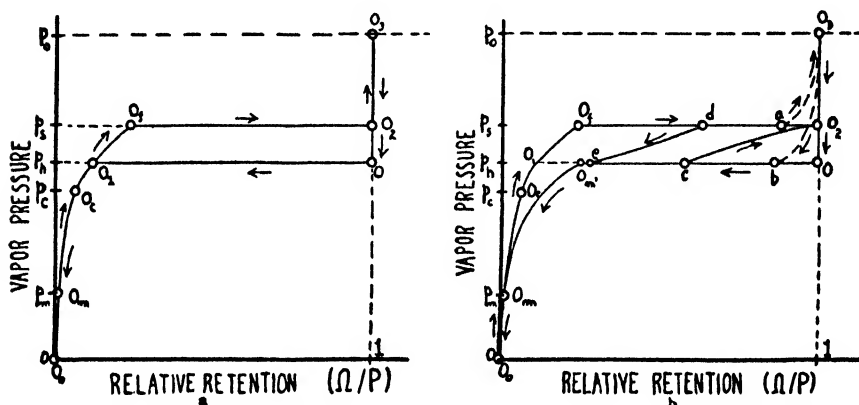


FIG. 4. SCHEMATIC CURVES SHOWING THE RELATIVE RETENTION REPRESENTED AS A FUNCTION OF THE VAPOR PRESSURE—THE SORPTION CYCLE

(a) Simple sorption cycle showing saturation hysteresis; (b) the distortions produced by microscopic hysteresis and the presence of a free gas in the packing, together with the effects produced by reversing, in a partially saturated packing, either hydration or dehydration.

capable of sustaining a meniscus until the latter occupies the position of maximum capillary rise, in which position the meniscus threads through the plane of centers of each cell (9). All curvatures from that of a plane surface for a vapor pressure  $p_0$  down to that prevailing at maximum capillary rise for a vapor pressure  $p_h$  can be accommodated. Whether the meniscus comes from capillary rise or whether it forms by condensation, when removed, its behavior will, as far as curvature adjustments are concerned, be the same; it will remain in the layer of grains where it initially rests as long as its curvature can adjust itself to that layer. If, for example, a U-system is used (7, fig. 1) and liquid is allowed to rise into the soil contained in one arm of the U and thus reach equilibrium at minimum rise (7), then when the free liquid surface is lowered in the liquid arm of the U the meniscus will remain undisturbed, except for slight changes in the grain layer where it happens to be, until the distance between

the two, meniscus and free surface, exceeds the height of maximum capillary rise. Likewise if a soil sample is saturated and the vapor pressure over the meniscus is decreased, liquid will evaporate with an adjustment of the meniscus curvature in the grain layer where it happens to rest. The process will continue until a value of the vapor pressure  $p_h$  is reached beyond which curvatures not available in the packing, for a continuous meniscus, are required; this is the position of maximum capillary rise. Further decrease of vapor pressure means evaporation, and the continuous meniscus must break up. On dehydration, therefore, the packing will be saturated as the vapor pressure changes from  $p_0$ , that over a free surface, down to the value  $p_h$  when the meniscus runs through the plane of centers of each cell; the vapor pressure may change through a rather large range ( $p_0 > p > p_h$ ) with a negligibly small loss of liquid. We observe that  $p_h$  the critical saturation pressure of dehydration is less than  $p_s$  the critical saturation pressure for hydration, for  $p_s$  corresponds to a meniscus intermediate to  $p_0$  and  $p_h$ . These relations are shown in figures 3a and 3b. If, therefore, the packing is dehydrated commencing at the point  $O_3$  in figure 4a, corresponding to  $p_0$  (for a plane surface), we pass, as the saturation pressure is decreased, through the point  $O_2$  corresponding to  $p_s$  the saturation pressure for hydration, and the curve  $O_3O_2$  is common to both hydration and dehydration. At  $O_2$  the curves of the two processes separate; the dehydration curve continues downward, with the packing saturated, until 0 the critical point of dehydration, with vapor pressure  $p_h$ , is reached. Values of  $p$  on the hydration curve, such that  $p_s > p > p_h$ , correspond to a discontinuous distribution of liquid masses; whereas those on the dehydration curve correspond to a saturated soil with the meniscus in the outer boundary. The value  $p_h$  is easily obtained; if we apply equation (E) to the boundary with the meniscus in the position of maximum capillary rise (9), we find for the critical saturation pressure of dehydration that<sup>3</sup>

$$p_h = p_0 e^{-\frac{\sigma D_0}{r \rho p_0} \frac{2}{[0.9590/(1-P)^{2/3}] - 1}} \quad (Z)$$

and for  $\lambda_h$  [equation (E) with  $p = p_h$ ] we have

$$r\lambda_h = 2/[0.9590/(1-P)^{2/3}] - 1 \quad (AA)$$

The continuous meniscus, when the vapor pressure is decreased below  $p_h$ , travels inward over the packing and disappears; discontinuous masses of liquid, broken off during the inward journey, then exist in the soil. The continuous meniscus may break up for either pendular or funicular vapor pressures, depending on the porosity of the packing. The values of equation (AA) range from  $r\lambda_h = 4.20$ , for a porosity  $P = 0.48$  (porosities may be greater than 0.48),

<sup>3</sup> Equation (Z) is obtained by applying equation (E) to the spaced hexagonal packing; the value  $(\int ds \cos \theta)/a$  is weighted according to the frequency of occurrence of the  $T$ ,  $S$ , and  $R$  cells of the unit element (9).

to  $r\lambda_h = 11.63$  for a porosity  $P = 0.26$ . When the value  $r\lambda_c = 6.464$  [from equation (O)], which marks the junction of the pendular with the funicular region, is substituted in equation (AA), the corresponding porosity is found to be  $P = 0.373$ . Hence if  $r\lambda_c < 6.464$ , in which case  $P > 0.373$ , the saturation meniscus, on breaking up, leaves funicular liquid masses. If  $r\lambda_c > 6.464$ , in which case  $0.373 > P > 0.26$ , the saturation meniscus disintegrates in the pendular region. Referring to figure 4a, we expect the water content to decrease greatly along  $O_0I_1$ , under a constant vapor pressure just less than  $p_h$  (the distance of  $O_0I_1$  from the  $\Omega/p$  axis).  $O_0I_1$  should meet the hydration curve  $O_0O_cO_f$  at  $O_1$  when the continuous meniscus has completely disappeared. We should then expect that, as the vapor pressure is further lowered, the funicular region  $O_1O_c$  would be retraced, thence the pendular region,  $O_c$ , to the origin  $O_0$  where the soil is completely dried. This is not the case, however, for, because of the existence of microscopic hysteresis (8), neither the funicular region nor the part  $O_cO_m$  of the pendular curve is reversible.

Microscopic hysteresis is confined to configurations composed of a small number of grains. The random tetrahedron, for example, formed by the centers of four neighboring grains may for a given vapor pressure, suitably chosen, support either single rings at its contact points or a web of liquid, or finally it may be saturated; each capillary surface presents the same curvature. The Kelvin equation is satisfied with the tetrahedron subject to the same vapor pressure in each case. The amount of liquid retained in the tetrahedron is, however, different; when rings exist it is a minimum, and when the cell is saturated, a maximum.

The reason for the hysteresis lies in the fundamental difference of mechanism in the two processes. In hydration all liquid masses are formed by growth and coalescence of simpler forms; the amount of absorbed liquid must be a minimum. In dehydration, on the other hand, the discrete liquid masses are broken off the continuous meniscus as it moves through the interior of the soil, and left in the soil; after they are once placed in the packing, alteration is produced solely by evaporation. The relative sizes of the pore cells in a small volume of packing are of importance; a small pore may accommodate the curvatures necessary to a meniscus extending over it, whereas more open surrounding configurations may not. If, for example, we consider a close regular tetrahedron, formed by four neighboring grains in contact, and its surrounding grain configurations supposedly sufficiently more open than the tetrahedron, then the main capillary meniscus, as it moves through the compound configuration, will leave a capillary surface extending across each of the four pore cells bounding the tetrahedron while the more open pore cells surrounding it will be left empty since they are too large to support similar capillary surfaces; the tetrahedron will remain saturated as long as possible. Thus isolated liquid masses, each of which completely saturates the grain configuration supporting it, may exist during dehydration, whereas, during hydration, the identical configurations may be unsaturated for the same vapor pressure.

There is a definite limit to the size of these liquid forms. As the vapor pressure is decreased, the capillary surfaces bounding the web (for example, the liquid mass filling the interior of a three grain pore) recede farther into the supporting cell, until they meet in its plane of centers; each liquid mass must then break into rings. The extreme case occurs when the configuration is saturated; each bounding capillary surface may recede until it occupies the smallest opening of the cell (e.g., the plane of centers of the regular tetrahedron) beyond which it must break into rings.

The limiting vapor pressures of microscopic hysteresis may be easily fixed. The extreme configuration present in the random piling of the soil is a close regular tetrahedron with six actual contact points and outer faces composed of four three-grain pores. Each contact point is capable of supporting a ring until these merge when [equation (G)]  $\lambda_c = 6.464/r$  and  $p_c = p_0 e^{-6.464(D_0 \sigma / r \rho p_0)}$ . The tetrahedron may be saturated until the bounding capillary surfaces occupy the smallest opening in each of its faces; i.e., the plane of centers, the position taken during maximum capillary rise into the tetrahedron. The vapor pressure at which this occurs can easily be found; it is sufficient to consider a single face and apply equation (D). We find if  $\lambda_m$  is the value of  $\lambda$  at which it occurs that  $\lambda_m = \pi r \sigma / (3^{1/2} r^2 - \pi r^2)$ , or

$$\lambda_m = 19.5/r \quad (BB)$$

Hence the limiting vapor pressure  $p_m$  is

$$p_m = p_0 e^{-19.5(D_0 \sigma / r \rho p_0)} \quad (CC)$$

Thus, during hydration, rings will exist in the tetrahedron when  $\lambda_c < \lambda < \lambda_m$ , whereas, during dehydration, it will be saturated in the same interval. When the vapor pressure becomes less than  $p_m$  the meniscus associated with the tetrahedron must break into rings, and no hysteresis is possible beyond the vapor pressure given by equation (CC). The dehydration curve is identical with the watering curve for values of  $p$  from zero to  $p_m$ ; the absorption isotherm is completely reversible for values of  $p$  such that  $0 < p < p_m$ .

The effect of microscopic hysteresis is to alter the dehydration curve shown in figure 4a. The main meniscus will disappear at some point  $0_m$  on the dehydration curve instead of at  $0_1$ , as shown in figure 4b; the water content is greater during dehydration, because of the presence of a number of saturated configurations not present in hydration, and by an amount indicated by the distance  $0_1 0_m$ . As the vapor pressure is lowered the dehydration curve must run to the right of the hydration curve, but, as the saturated configurations disappear, approach closely to it. The saturated configurations become unsaturated at different vapor pressures, for they are in distribution depending on the porosity (the ideal soil is a random piling); and since the number of close tetrahedrons is small, deviations of the two curves should not be great near  $\lambda_c = 19.5/r$ . The dehydration curve must finally meet that of hydration at  $0_m$ , the limit of



microscopic hysteresis. From there on, as the vapor pressure is decreased, the two curves are identical and are represented by  $0_0 0_m$ .

Figure 4b shows the complete cycle for capillary condensation in an ideal soil.  $0_0 0_m 0_2 0_3$  is the hydration curve, and  $0_3 0_2 0_1 0_m 0_0$  is that of dehydration. The existence of saturation hysteresis is clearly shown. The soil may exist completely saturated for any vapor pressure  $p$  such that  $p_h < p < p_s$ . The entire region of hysteresis is confined between the vapor pressure  $p_m$  for the junction  $0_m$  of the two curves in the pendular region, and the vapor pressure  $p_s$  for  $0_2$  the junction in the saturation region. Both curves are identical in the

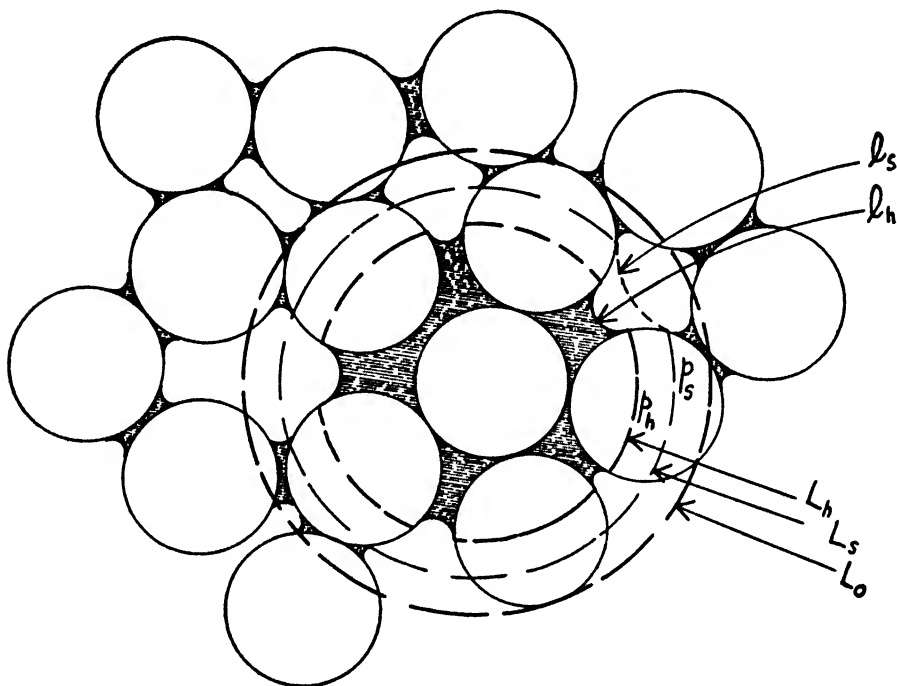


FIG. 5. SMALL PACKED SAMPLE WITH MENISCUS CONTRACTED TO THE INTERIOR OF THE PACKING

The notation used is identical with that of figure 3

region  $0_0 0_m$  and  $0_2 0_3$ . In the hysteresis region there are two types of hysteresis, saturation and microscopic; the latter alone occurs when  $p_m < p < p_h$ , whereas both types may occur when  $p_h < p < p_s$  (in the funicular region). On the curve  $0_0 0_m$ , for example, the part of the packing over which the meniscus has passed contains the small saturated configurations characteristic of microscopic hysteresis while the remainder is saturated; on the corresponding curve for hydration  $0_2 0_3$  the same configurations may be unsaturated.

In the saturation region ( $p_h < p < p_s$ ), we may cross from the curve  $0_m 0_0$  to  $0_2 0_3$  and *vice versa*. Such a crossing would occur in a partially saturated pack-

ing. If we start at the point  $c$  on  $0_m \cdot 0$ , we recognize that the capillary-condensed liquid is in two distinct capillary states; first, in a bulk saturating part of the packing and bounded by the original capillary meniscus, now contracted to the interior of the packing; and secondly, as discrete masses existing in the remainder of the packing and formed as the main meniscus passed over the region; these relations are shown in figure 5. If we hydrate, both masses will grow; the main meniscus will merely adjust itself to the layer of grains where it happens to rest, and only a small amount of liquid will be absorbed, the process being similar to that on  $0_2 0_3$ ; the discrete masses will grow by condensation and, when the vapor pressure  $p_s$  is reached, will completely combine. In general, we should reach the curve  $0_f 0_2$  at the point  $0_2$ . The water content, for a given vapor pressure, along  $c 0_2$ , in a packing which has been partially dehydrated to a point such as  $c$  and then re-hydrated should be greater than if the packing had been subjected only to hydration. On the curve  $c 0_2$  the water content is the sum of two bulks, one saturating part of the packing and the other composed of funicular masses embedded in the remaining part. The water content should be greater by approximately the difference, in the saturated portion, between the volume of the pore space and the volume of the funicular masses filling it at the same vapor pressure (Fig. 5). Hence, in general, the intermediate curve should meet  $0_f 0_2$  at  $0_2$ , the point of complete saturation. If, on the other hand, we stop hydration at some point  $d$  on  $0_f 0_2$  and dehydrate, we then trace another curve  $de$  which represents the breaking up of the discrete masses which exist on  $0_f 0_2$  at the point  $d$  (complete coalescence has not occurred, and does not occur, until  $0_2$  is reached). The curve  $de$  will not be identical with  $0_m 0_d$  because of microscopic hysteresis in the funicular region; many saturated configurations exist at  $d$  formed by prior coalescence and they preserve their forms during dehydration as long as possible. The curve  $de$  should finally intersect  $0_m 0_m \cdot 0$  at  $e$  somewhere near the boundary of the pendular and funicular regions.

The foregoing discussion of the absorption cycle has rested on the supposition that only the liquid and its vapor are present in the soil. Should a gas soluble in the liquid be present, the matter is more complicated, especially at vapor pressures near those of saturation. When the soil is hydrated, the mergence of funicular masses may occur in such a way that small bubbles of gas are trapped and held enclosed in the supporting grain configuration by the newly coalesced liquid. The gas, air for example, may remain thus enclosed for a long time. The hydration curve will be greatly modified near the saturation region ( $0_2 0_3$ , fig. 4b).

The presence of a meniscus disturbs the normal internal pressure of the liquid and must be considered (11). The pressure just over the meniscus is  $p$ , that in the interior less by an amount  $\Delta p$  where  $\Delta p$  depends on the curvature (Laplace equation). If the vapor pressure  $p$  over a funicular liquid mass, belonging to the hydration curve, for example, is increased, the curvature of the funicular meniscus must decrease;  $\Delta p$  then decreases and the interior pressure increases toward  $p$ ; thus gas enclosed in the funicular liquid mass will be compressed and

tend to go further into solution. After complete coalescence has occurred and a continuous meniscus has formed at the saturation pressure  $p_s$ , the process will continue until the normal vapor pressure  $p_0$  is reached. The hydration curve will be distorted; at some point  $a$  on  $0_10_2$  the actual curve will leave  $0_10_2$  and follow a curve  $a0_3$  (broken line in figure 4b) instead of  $a0_20_3$ . The actual curve may not, in some cases, even meet  $0_20_3$ . The reverse process accompanies dehydration. If the vapor pressure  $p$  over the meniscus is decreased, then the curvatures and  $\Delta p$  also will increase, with the result that the interior pressure becomes less; gas dissolved will come out of solution, and the soil will contain many microscopic gas bubbles, the volume of which depends on  $p$ . The soil, although apparently saturated, is not saturated by the volume of gas released in the packing. The normal dehydration curve  $0_30_20b$  becomes replaced, actually, by the curve  $0_3b$  (marked by a broken line in figure 4b).

If the soil sample is sufficiently large, the distribution of the capillary-condensed liquid will be influenced by gravity and it will no longer be homogeneous. If, however, the vapor pressure is known at one single point of the packing, the distribution can be calculated by a simple extension of methods given in another paper (8). If, for example, the vapor pressure  $p_A$  is known for a point  $A$  at the top of the soil, that at a point  $H$  distant from  $A$  by  $h$  cm. is<sup>4</sup>  $p_H = p_A e^{(D_0 g h / p_0)}$ . It is then possible to determine the capillary state at any point in the soil in terms of the single vapor pressure  $p_A$  and the distance  $h$ . Since  $\log_e (p_H / p_0) = \log_e (p_H / p_A) + \log_e (p_A / p_0)$  we have from equation (G)

$$\lambda = -\frac{\rho p_0}{\sigma D_0} \left[ \frac{D_0 g h}{p_0} + \log_e \frac{p_A}{p_0} \right] \quad (DD)$$

to be used in equations (M) to (CC) when determining the capillary state and quantity of liquid present in the soil. It is easily seen that all three zones of capillary distribution may be present if the soil column is of sufficient length. The vapor pressure at the reference point  $A$  and the length of the soil column determine which capillary zones of distribution are present. The problem is identical with that discussed in a paper (8) on the distribution of liquid in soils. The long soil column may be regarded as a superposition of a number of small samples each with a different vapor pressure; the distribution in each sample is homogeneous but varies from sample to sample. The distribution for an infinitely long soil column is identical with that shown in figures 4a and 4b. Figure 6 shows the relative retention represented as a function of the vapor pressure and also as a function of the distance  $h$  of a given soil lamina above a plane liquid surface. In an actual soil only part of the distribution there shown will be found, and the appropriate part of the curve will depend entirely on the range of vapor pressures prevailing in the soil. Figure 4b gives a more correct

<sup>4</sup> From the relation  $h = - \int_{p_A}^{p_H} D g dp$  and from Boyle's law  $D/D_0 = p/p_0$  we find  $\log_e (p_H/p_A) = D_0 g h / p_0$ , from which is found the value given above;  $h$  is measured downward from the line  $p_A = \text{const.}$

picture of the complete distribution, since disturbing effects such as microscopic hysteresis are shown.

So far, the discussion has been restricted to capillary condensation in the ideal soil. But, in general, both capillary condensation and surface adsorption are present. One, however, may be negligible in comparison with the other; the grain size is the controlling factor. With coarse soils composed of larger grains, capillary condensation is sufficient to account for the absorbed liquid. When, however, the grains approach colloidal size, surface adsorption cannot be neglected and can, especially at small vapor pressures when capillary condensation is negligible (very small rings), account for the greater part of the absorbed vapor.

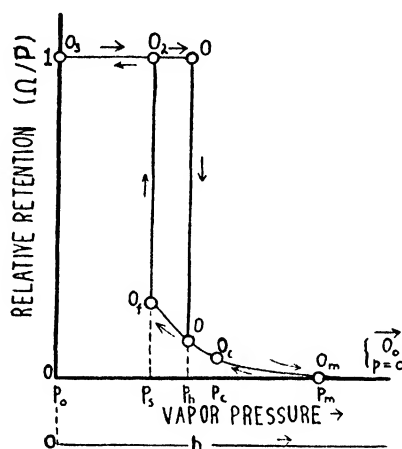


FIG. 6. SCHEMATIC DIAGRAM OF THE POSSIBLE MOISTURE DISTRIBUTIONS IN A LONG SOIL COLUMN

The relative retention is represented as a function, first, of the vapor pressure prevailing in a given lamina; and secondly, of the distance  $h$  of the lamina from a free liquid surface (the notation is identical with that of figure 4).

Surface adsorption occurs only on the parts of the grain surface not covered by capillary condensed liquid; for example, there is no surface adsorption in the saturation region, represented by  $0_20_3$  of figure 4b. It occurs principally in the pendular and funicular regions. It will, where appreciable, displace the curves  $0_00_m0_c0_f$  and  $0_m0_m0_0$  of figure 4b to the right; the total retention is the sum of the capillary condensed liquid and the amount held by surface adsorption. It is, for a single grain, equal to the area of a sphere-surface,  $4\pi r^2$ , less the total area of all the polar caps of the grain covered by rings. The area of a polar cap is  $2\pi r^2(1 - \cos \theta)$  (figure 7) where  $\theta$  is the angle of the ring. If there are  $n$  contacts per grain and each supports a ring of liquid, the net area  $b$  will be given by

$$b = 2\pi r^2[2 - n(1 - \cos \theta)] \quad (EE)$$

We have shown elsewhere (6) that  $n = 6(1 + 1.828 x)/(1 + 0.414 x)$  where  $x = (0.476 - P)/0.217$ ; from equation (H) we have  $\cos \theta = 1/(1 + 1/r\lambda)$ ; hence, with  $\lambda$  given by equation (G), the surface available at any particular vapor pressure in the pendular region can be calculated. The total surface  $B_p$  available in 1 cc. of packing is  $B_p = mb$  where  $m$  is the number of grains per cubic centimeter and given by  $m = (1 - P)/[(4/3)\pi r^3]$  or the equivalent expression  $m = (1 + 0.414 x)/8r^3$ . To find the total surface adsorption we must know

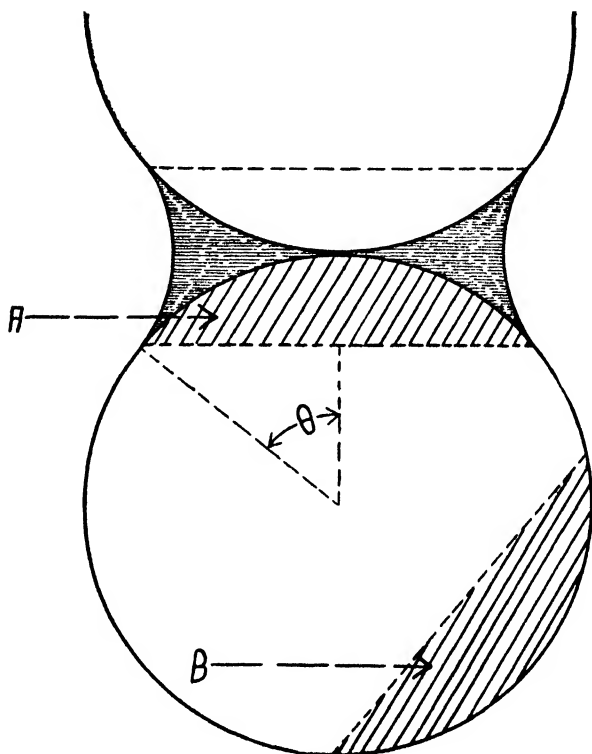


FIG. 7. SORPTION PHENOMENA ON A SINGLE GRAIN

The shaded areas A and B show portions of the sphere-surface covered by capillary-condensed rings; the remainder of the grain surface is available for surface adsorption ( $\theta$  is the angle defining the size of the ring covering the segment A).

the concentration  $c$  (mass of vapor adsorbed on unit area) on the surface for the particular vapor pressure and temperature in question. The total liquid  $\omega_p$  held by surface adsorption will be given by

$$\omega_p = cmb \quad (FF)$$

We may proceed similarly in the funicular region, if, as discussed earlier, minimum capillary condensation is involved. The area  $b$  available for surface

adsorption on one grain is given by equation (EE). For the average packing used in the funicular region (6), however,  $n = 12$ ;  $\cos \theta$  is, from equation (Q), given by  $\cos \theta = q/[1 + 1/(r\lambda)]$ , with a value of  $q$  obtained from equation (R) and  $\lambda$  from equation (G). The total available surface per cubic centimeter of packed space is  $B_f = mb$  where  $m = (1 - P)/[(4/3)\pi r^3]$ , and the total adsorption in terms of the concentration  $c$  is

$$\omega_f = cmb \quad (GG)$$

The total retention  $\Omega'_p$ , for the pendular region, is the sum of the surface adsorbed ( $\omega_p$ ) and capillary condensed ( $\Omega_p$ ) vapor, obtained respectively from equations (FF) and (M), or

$$\Omega'_p = \omega_p + \Omega_p \quad (HH)$$

Similarly for the funicular region, we have

$$\Omega'_f = \omega_f + \Omega_f \quad (II)$$

where  $\omega_f$  and  $\Omega_f$  are, respectively, the actual amounts of surface-adsorbed and capillary-condensed liquid in the funicular region.

It appears from this discussion that capillary condensation is, for most ideal soils, chiefly responsible for the sorbed liquid. Surface adsorption is important and need be considered only when the grains approach colloidal size ( $10^{-6}$  cm.). A simple calculation will show that this is the case; we regard the adsorbed water as spread on the available grain surface at a thickness of one or two molecules and at unit density or even double that value. Actual soils are a mixture, and the predominant sorption process will be determined by the amount of colloidal matter present.

If the soil material is transparent, and is to absorb the vapor of a clear liquid, the optical behavior of the system is an important phenomenon. If the refractive indexes of the solid and liquid are close together, we may, as long as the soil is completely saturated, expect transparency. When, however, there is an unsaturated condition and rings or funicular masses of liquid are present, diffuse reflection from the capillary surfaces of such masses, especially those on the boundary, ought to render the soil opaque. Interference phenomena, no doubt, occur with soils composed of small grains. There should thus be a sharp optical transition on the dehydration curve, when, at the critical vapor pressure  $p_*$ , the distribution changes from saturation to one of discontinuous masses; the soil changes from a state of transparency to one of opacity. As the vapor pressure is lowered, the opaqueness should gradually disappear and the soil should be completely transparent when dry at  $p = 0$ . There should be a second transition, for hydration, when, at the critical vapor pressure  $p_*$ , the reverse change takes place; the soil should become gradually opaque and

then transparent when  $p > p_s$ , unless the saturated state is disturbed by the presence of a large amount of free gas.

The process of sorption, it is believed, is responsible for the slow transfer of soil moisture; a distillation or condensation takes place, generally, from or on the existing capillary liquid masses until the requirements of the Kelvin relation are satisfied. In that case, definite vapor pressures ought to be associated with the equilibrium of a long soil column. Table 1 shows the magnitude of the various vapor pressures to be expected for the sands used by King (4, p. 85-95; 8) in his retention experiments. Equation (C) has been used to compute these results; the necessary formulas can be established by following the procedure used for equation (D) of the text. These sands are comparatively coarse, and the vapor pressures differ but little. It must be remembered that these vapor pressures are for the final equilibrium state and that the time taken by a process of condensation in attaining them may be considerable.

TABLE 1  
*Summary of vapor pressures associated with King's experiments\**

	SAND NUMBER				
	20	40	60	80	100
Porosity.....	0.389	0.401	0.408	0.406	0.398
Radius of grains (cm.).....	0.02373	0.00924	0.00776	0.00592	0.00413
$(p_0 - p_s)$ dynes/cm. <sup>2</sup> .....	0.167	0.415	0.484	0.638	0.949
$(p_0 - p_h)$ dynes/cm. <sup>2</sup> .....	0.233	0.568	0.656	0.870	1.310
$(p_0 - p_o)$ dynes/cm. <sup>2</sup> .....	0.251	0.642	0.762	1.002	1.436
$(p_0 - p_m)$ dynes/cm. <sup>2</sup> .....	0.754	1.935	2.305	3.022	4.330

\* Water is used in these experiments;  $\rho = 1$ ,  $\sigma = 72$  dynes/cm.,  $p_0 = 1.27$  cm. of Hg,  $D_0 = 1.274 \times 10^{-6}$  gm./cm.<sup>3</sup>

The literature, finally, contains, as far as can be ascertained, no record of experimental studies of sorption in ideal soils composed of larger grains; that such should be made is desirable. The glass pearls used by Green and Ampt, also by Haines (2) furnish a realizable ideal soil. With the technique used by McBain, Anderson, Patrick (5), and others in their work on the gels of silicic acid, experimental studies of sorption cycles in an ideal soil ought to offer little difficulty.

#### APPENDIX I

Equation (A) is obtained as follows: We suppose the liquid and its vapor in a closed system to be in equilibrium and consider a capillary surface in communication with a free liquid as in figure 8. We let  $p_0$  be the vapor pressure just over the free liquid,  $p_a$  and  $p_{a'}$  the pressures just over and under an element  $dS$  of the capillary surface ( $a$  and  $a'$  are respectively points immediately above and beneath the element);  $h$  is the height of the element  $dS$  above the free

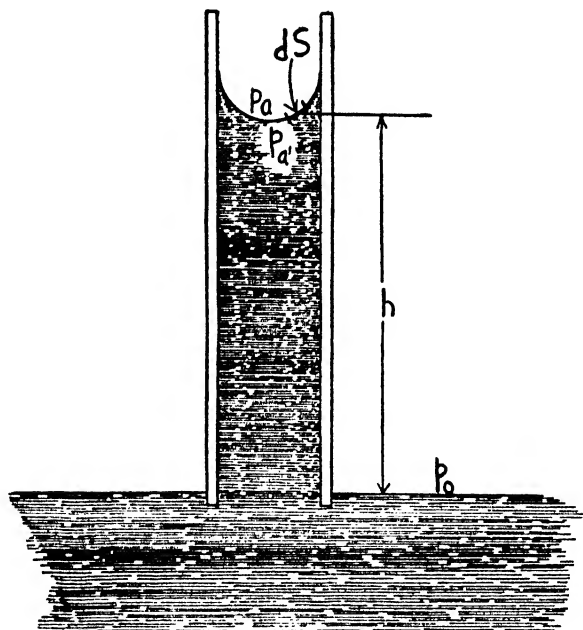


FIG. 8. SIMPLE CAPILLARY TUBE USED TO DERIVE EQUATION (c) OF APPENDIX I

liquid of density  $\rho$  and surface tension  $\sigma$ . We have then, if  $D$  is the density of the vapor at the height  $h$ , and  $g$  the acceleration of gravity,

$$p_0 = p_a + \rho gh$$

$$p_0 = p_a + \int_0^h D g dh$$

If we set  $\Delta p = (p_a - p_a')$ , we find

$$\Delta p = \rho gh - \int_0^h D g dh$$

Now  $dp = -Dgdh$  and  $h = - \int_{p_0}^p dp/gD$ ; also, Boyle's law being assumed,  $D/D_0 = p/p_0$ ; hence we find  $-\int_0^h Dgdh = (p - p_0)$  and  $h = -(p_0/gD_0)[\log_e(p/p_0)]$  where  $p_0$  is the vapor pressure and  $D_0$  the vapor density just over the free liquid. Therefore

$$\Delta p = -(\rho p_0/D_0) \log_e(p/p_0) - (p_0 - p) \quad (a)$$

The Laplace capillary equation is

$$\Delta p = \sigma(1/R_1 + 1/R_2) \quad (b)$$



where  $R_1$  and  $R_2$  are the principal radii of curvature of the element  $dS$  of capillary surface. Hence from equations (a) and (b) we have finally

$$\sigma(1/R_1 + 1/R_2) = -(p_0 - p) - (\rho p_0/D_0) \log_e(p/p_0) \quad (c)$$

Equation (c) is sufficiently general for most purposes and can be shown, by thermodynamical considerations, to hold for a capillary surface not communicating with a free liquid, just as Kelvin has done (8). It is applicable to either a concave or a convex surface. If, for example, we consider a simple capillary tube immersed in a liquid, its radius, to a first approximation, is easily found; from equation (c), if  $R$  is the radius of the tube, we have, since  $R = R_1 = R_2$

$$R = (2\sigma D_0)/\{\rho p_0 \log_e(p/p_0) - D_0(p_0 - p)\}$$

which is the equation derived by Anderson (1) also by Hückel (3, p. 237). If  $(p - p_0)$  is negligible, as is generally the case (3, p. 237), we have the usual form of Kelvin equation

$$\sigma(1/R_1 + 1/R_2) = -(\rho p_0/D_0) \log_e(p/p_0) \quad (d)$$

From equation (c), if  $p$  does not differ much from  $p_0$ ,  $\log_e(p/p_0)$  may be replaced by  $(p - p_0)/p_0$  and we find the form originally given by Kelvin (10)

$$\sigma(1/R_1 + 1/R_2) = \frac{(\rho - D_0)}{D_0} (p - p_0) \quad (e)$$

It is not difficult to obtain an integral form of equation (c), which is often more convenient for approximate calculation. We may multiply both sides of equation (c) by  $dS \cos \phi$  and integrate; we obtain equation (A) of the text.<sup>5</sup>

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<sup>5</sup> For the partial integration  $\int (1/R_1 + 1/R_2) dS \cos \phi = \int \cos \theta ds$ , see any treatise on capillarity, e.g., Weber and Gans, "Repertorium der Physik."

# EROSION CONTROL WITH THE S.E.R.A.

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In the spring of 1935 a study of soil erosion and its control was undertaken as a work relief project by the Monterey County branch of the California Emergency Relief Administration, with the writer as project supervisor.<sup>1</sup> The work consisted of two phases: first a survey of erosion conditions in the supervisorial district chosen for the project, and secondly, the setting of plants and the placing of structures designed to control or reduce erosion.

## EROSION SURVEY

The survey was made by 2-man crews whose members were chosen on the basis of their knowledge of the locality, ability to make reports, etc. A total of 325 farms were visited, representing about one-third of the area of the district. Of the rest, some land was essentially level and some in a limestone section, neither type of land presenting a serious erosion problem. The soil of the section studied is prevailing sandy, some wind-borne, some residual from soft sandstone bedrock.

<sup>1</sup> For this work a nominal crew of 21 men was allotted, and a period of 8 weeks was allowed. The Monterey County Supervisors acted as sponsor for the project, providing office supplies, assessor's maps of the district, transportation for the project supervisor, etc.

The workers themselves were local men (the typist, a man or a woman) of varied ages and background, but mainly with agricultural experience. The compensation was based on a budget established individually for each worker and determined by his needs for rent, food, etc. Generally speaking, the man with the bigger family had a larger budget. The man was given enough work (in a 4-week month) to earn the amount of his budget. If, for example, his budget was \$42 and the rate of pay \$.50 an hour, his "work-ticket" would call for 84 hours' work, or 14 6-hour days. The work week was 5 days. Thus it was not uncommon, but rather the rule, that a man did not work the entire month (20 days). A man might be laid off for a week or two just as he had become well trained or demonstrated his fitness for the work in hand. These irregular labor conditions, however, caused no serious interference with the progress of the work.

Credit for the successful prosecution of the project must be ascribed largely to the interest and industry of the members of the crew, especially to two successive straw-bosses, Opel Wood and S. S. Gordon, of Aromas. Reports on the work done were prepared by S. S. Gordon and by Cyril Kirby. The project supervisor acted chiefly as a catalyzer. Grateful acknowledgment is also made of helpful suggestions given by Watsonville and Santa Paula members of the U. S. Soil Erosion Service (now known as the Soil Conservation Service).

Excluding the supervisor's time, the prosecution of this project cost 535 man-days of work distributed as follows: survey, 78; control measures, 441; typist, 16.

The chief object of the survey was to ascertain the magnitude of the erosion problem in the district. A summary of the information obtained is presented in table 1. Discrepancies in the totals of this table are due to lack of replies on the part of some of the farm owners or renters.

About one-seventh of the farms visited reported a reduced use of the land because of erosion. In many cases this reduction consisted merely in untilled patches left in the cultivated fields because of the difficulty of running plows and cultivators across gullies or because of the risk of causing further erosion. In a more aggravated stage, cultivated land was abandoned, wild plants were allowed to grow up, and the area was used for pasture or even left unutilized in brush.

In the case of reduced crop yields, it was obviously impossible to separate reductions due to erosion from those ascribable to other causes. For about

TABLE 1

*Summary of information obtained in erosion survey, District 1, Monterey County, California, February-April, 1935*

Amount of erosion and control

No erosion.....	121 farms	Control practiced by owner.....	86 farms
Little erosion.....	133	Control by owner unnecessary	
Much erosion.....	65	or neglected.....	224
Total.....	319	Total.....	310
<hr/>			
Land use reduced because of erosion.....			47 farms
Land use not reduced by erosion.....			266
Total.....			313
Crop yields less than earlier yields.....			158 farms
Crop yields not notably less than in former years.....			119
Total.....			277

half the farms visited, lower yields were reported, but the most we can say is that in some of these instances soil erosion was undoubtedly the main cause.

The small amount of clayey soil in this section is found mainly in level patches and is thus not subject to washing; but where it occurs on a moderate slope the erosion, as would be expected, is less than on a similar slope with sandy soil.

In the survey the land was characterized as steeply sloping, moderately sloping, or level. The amount of vegetative cover was also noted. The information obtained does not indicate much correlation between these factors and the amount of erosion. Most of the farms studied were situated on moderate slopes. Erosion was reported as varying from none to considerable on these areas, both bare and with good vegetative cover. In the relatively few cases where a timber or brush cover was noted, however, erosion was re-

ported to be inappreciable. On the other hand, one or two farms situated on steep slopes with sandy soil were reported to be relatively free of erosion.

For the purpose of determining suitable plants for vegetative control of erosion, a note was made of the important native vegetation on each farm visited. The designation "native" must be given a very broad interpretation in this connection, for the three species most commonly observed are all naturalized European plants; namely, filaree (*Erodium cicutarium* and perhaps *E. moschatum*), mustard (*Brassica campestris*), and wild radish (*Raphanus sativus*). Filaree, a rosette plant in youth, belongs to the geranium family, and the other two are fast-growing members of the mustard family. All three are annuals (the radish may also be a biennial). These luxuriant crops are reputed to afford excellent protection to the soil (either as volunteers or sowed), but were not usable in the S.E.R.A. work. Filaree, incidentally, is a valuable forage plant. On abandoned land coyote brush (*Baccharis pilularis*, Compositae) is one of the first invaders. It would undoubtedly be useful on raw slopes, but there is a local prejudice against it as a troublesome weed. In orchards its presence is regarded as a sign of neglect.

Certain current agronomic practices have a good deal of bearing on the erosion problem in this section. The extensive raising of peas, for example, promotes soil losses. In the past, when oats were more commonly raised in this section than now, the ground was kept fairly well covered, and cultivation was not practiced after sowing. Peas, however, now a more profitable crop than oats, are spaced farther apart and cultivated after each rain. The soil is thus left in ideal condition for eroding when the land has any appreciable slope. Again, it is locally considered good practice, because of the dry summer season, to keep the ground bare under and between orchard trees at this time so that valuable moisture will not be lost through transpiration by other plants. As a result, considerable erosion occurs in the orchards of the section with the advent of the fall rains or when a late spring rain follows spring cultivation.

Undoubtedly changes in farm management would result in an astonishing improvement in the erosion situation in this section, as in many other agricultural regions. It is also unquestionably true that some of the land here is bound to erode under almost any system of cultivation and should never have been cleared of its native growth in the first instance. That, however, is more easily observed than remedied. There are places where the use of other crops than the present ones would be far better for the farmland, but the other crops do not bring in satisfactory returns. One promising ground-covering crop, however, is worth mentioning, namely eucalyptus. There are several groves of these trees in the district, located on ridge tops where the sandstone bedrock is near the surface and cultivation is utterly out of the question. Harvested at the age of 15 years for fuel wood, this is said to be a surprisingly profitable crop, comparing very favorably, on the basis of annual returns, with other crops of the region. The wood seems to be well liked for

cooking and heating stoves. The second crop is a coppice stand, which, if properly thinned early in life, will yield an appreciable amount of pole, post, or fuel wood.

Besides the deterioration of agricultural land, soil erosion has another very important aspect, namely, the blocking of roads by sand carried down from the adjacent slopes. It is this phase of the problem which is of most concern to the county engineer. Sand removal is a costly annual item. Unfortunately the remedy is not to be found in such a program as ours, but rather in improved agricultural practice. Sometimes faulty location of a minor road is responsible for the troublesome sand which washes down to a more important road.

#### CONTROL MEASURES

The efforts made to control erosion were admittedly of an experimental nature, but were based on the teachings and practice of the U. S. Soil Erosion Service, modified by local conditions and experience. We were well aware in this work that we were attacking only the middle phases of the problem. On the one hand, we were not in a position to do much about the insidious sheet and shoestring erosion, which, though less spectacular than the familiar gully erosion, is none the less serious in reducing the value of agricultural land. The control of such erosion is mainly a matter of farm management. On the other hand, we were unable to install any of the more elaborate control structures of rock, masonry, concrete, or timbers, such as the Soil Erosion Service is building on its experimental areas. The object of the S.E.R.A. work was to pay *wages*, and money for *materials* was accordingly limited in amount. There is a question, too, as to whether the requisite skilled labor would have been available on the local relief rolls, even if it had been possible to obtain more materials. As it was, most of our work was done with materials which could be had for the asking, largely willow from the stream bottoms and the similarly ubiquitous haywire.

The following control measures were undertaken:

Planting of willow cuttings and sea-fig (*Mesembryanthemum aequilaterale*, locally known as "beach-apple").

Construction of nearly 200 soil-saving and check dams.

Construction of half a dozen "brush stairways."

Placing of brush on eroding slopes and in narrow gullies and at gully heads.

Laying of 180 feet of 12-inch concrete drain pipe.

Setting of sand bags.

Digging of diversion ditches.

Several variant types of dam were tried, mostly constructed of willow in some form or other. In a few cases small logs, up to about 6 inches in diameter, were wired to upright willow posts and the ends set well into the bank. Although these proved fairly effective, especially for relatively high dams in narrow gullies, they made such heavy demands upon the available material that it was impracticable to employ them on an extensive scale.

Employing smaller material is the wattle (or woven) dam, a type of structure to which willow is admirably adapted. For these dams, also, willow posts were used. These two dams are essentially the same as those pictured by Ramser.<sup>2</sup>

Another type was developed by the crew in the course of the work. This consisted of a double row of posts with brush between. The posts were spaced  $1\frac{1}{2}$  to  $2\frac{1}{2}$  feet in the row, with the rows a foot or two apart, mostly 15 or 16 inches. The brush was tramped down; the posts were wired together in pairs, the wire extending across the brush; and finally the posts were driven deeper to compact the brush. The result is a sort of brush wall 2 to 4 feet high, mostly 3 feet. Presumably this kind of dam has been used elsewhere, but A. B.

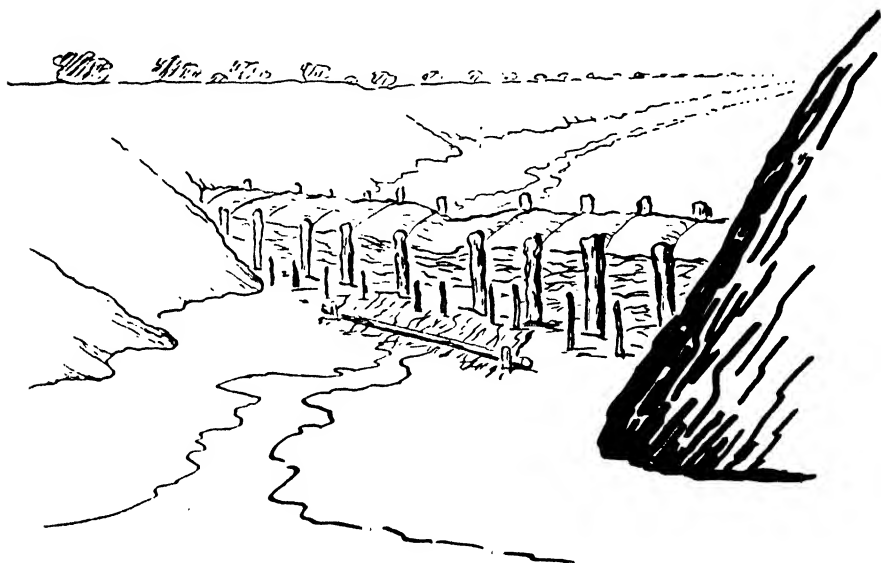


FIG. 1. "HARRIS" TYPE DAM IN PLACE, WITH APRON AND ROW OF CUTTINGS ON DOWNSTREAM SIDE

Harris was the originator of it on our work, and his fellows referred to it as the "Harris" dam. It seems to be a very effective model and in our later work was used to an increasing extent. It is illustrated in figure 1.

Another kind of brush dam was made with a broad base and narrow top. After the brush was piled in a windrow across the gully, pairs of posts or stakes were driven into the ground at an angle, one on the upstream side, the other downstream. Where these crossed above the brush, like saw horse legs, they were wired together and subsequently were driven deeper, thus compacting the brush mass. From its appearance we spoke of this type as the "sawbuck"

<sup>2</sup>Ramser, C. E. 1925 Gullies: how to control and reclaim them. U. S. Dept. Agr. *Farmers' Bul.* 1234: 15, 17.

or "rat's-nest" dam. It proved to be one of the most effective kinds for catching sediment.

A few dams were made of hog wire or chicken wire fastened to redwood pickets or willow posts.

In some of the smaller gullies, brush of various sorts, thoroughly tramped in, was used to good advantage. With brush of smaller caliber, stakes were used to keep it in place. In narrow, steep gullies, these brush fills sometimes took the form of crude dams with bare spaces between the dams.

A further development of this control method was the "brush stairway," as the men aptly called it. This type of structure was employed in some of

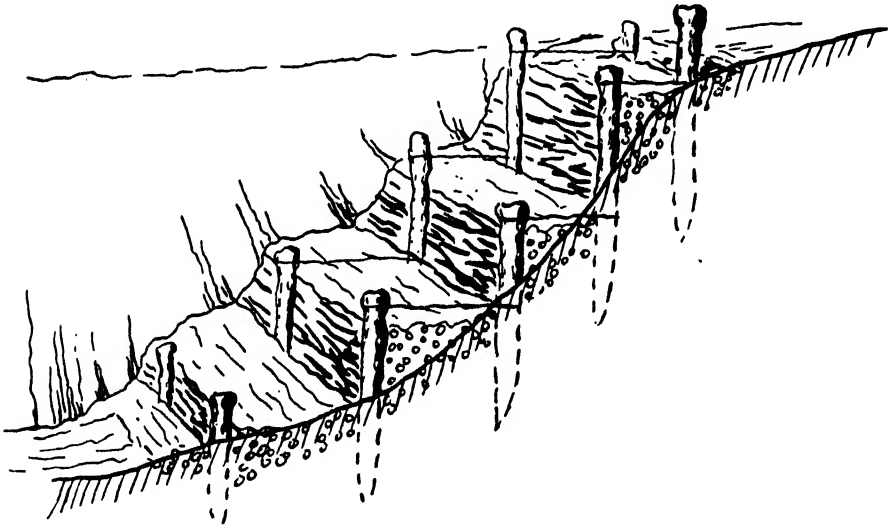


FIG. 2. "BRUSH STAIRWAY" IN NARROW, STEEP GULLY

the steeper gullies, often narrow laterals extending into cultivated land on the sides of an older, larger gully. It consists of masses of brush (of willow or of orchard prunings) placed in the gully one above another like a flight of steps, the front of each block of brush set against a line of posts deeply driven into the soil a foot or two apart. Sometimes the posts of one step were wired to those of the next step above. Depending on the width and pitch of the gully, the brush steps were made with treads up to 4 feet deep and 7 feet wide. The risers might be 2 feet in places. This structure is illustrated in figure 2.

When any obstruction is placed in a water channel there is the risk that the water will erode the earth around or under the obstruction. It is for this reason that the casual dumping into a gully of miscellaneous trash, such as

orchard prunings, old stoves, worn-out bedsteads, and wrecked automobiles, often proves nearly or quite valueless in checking erosion. All dams and other barriers should preferably be set well into the walls and bottom of the gully and should be lower in the middle than at the ends. In the case of a dam with vertical face, it is essential to provide some sort of an apron to prevent undercutting by the falling water on the downstream side. In our work we used for this purpose willow branches laid parallel to the direction of water flow, adjacent to the lower face of the dam, and held in place with a cross pole, as recommended by Ramser<sup>3</sup> (fig. 1).

The planting consisted mainly of willow cuttings. These were of various lengths and diameters, running 3 or 4 feet long set with half a yard extending above ground. Some were as much as 2 inches in diameter. Where willow twigs were available in quantity and the soil permitted, the cuttings were set a foot or so apart just below a dam, or sometimes both above and below (fig. 1). The posts of the dams were also usually of willow 2 or 3 inches in diameter.

Examination in the fall of 1935 disclosed a gratifying amount of new growth on willow cuttings, posts, and even some of the horizontal wattles. In a few cases willow posts bore sprouts 6 or 8 feet tall (in October). In some instances sprouts on posts and cuttings had died after reaching a length of a yard or so. It is to be assumed that there was a corresponding root development, which of course would help to hold in place the sand of the gully bottom. Possibly, too, the roots are still living and will send up new sprouts when the rains begin. In one large gully, 10 or 20 feet deep and 40 to 75 feet wide, the cutting of willows for dam construction resulted in a dense growth of sprouts 2 or 3 yards tall in the spaces between the dams.

In a few places sea-fig was used. Although this rapidly growing, decumbent, native succulent (of the family Aizoaceae) is employed locally to a considerable extent on cut banks along the roads, some farmers object to its use in cultivated fields because of a fear that it will spread as a troublesome weed. Judged by the apparent ease with which it is kept under control adjacent to cultivated orchards in some parts of the district, this apprehension is probably groundless. Nevertheless it is something to be reckoned with in planning control work.

On one particularly bad gully with slipping sides, both slopes were planted with sea-fig cuttings a foot or two long spaced one foot apart. By the middle of August these cuttings had made satisfactory growth, with an encouraging percentage of survival.

Since there was no publicly owned land available for our experiments, the control work was done on private land after the owners' permission had been obtained. As a general policy, there is definite objection to spending public money for projects benefiting only a few individuals. In view, however, of

<sup>3</sup> Ramser, *l. c.*, p. 15.



the need for experimentation and the educational value of the work, the use of private land was considered warrantable in this instance.

On the whole, the work done can be regarded as successful. The dams and brush fills have for the most part caught water-borne sand; willow cuttings and posts have sprouted and unquestionably developed fairly extensive root-systems; sea-fig is growing well; and the brush fills seem to favor the growth of blackberries and other vines. Before final judgment is passed on the effectiveness of the measures taken, at least a year should be allowed to elapse. It is to be kept ever in mind, too, that such work cannot be done once and for all and then left to itself. The control of erosion requires constant watchfulness, and control structures must be kept in repair and augmented as needed. This is not an S.E.R.A. task.

Although the primary purpose of the project was to provide relief work, yet it was hoped that our activities would serve to stimulate interest in soil conservation in Monterey County as in the case of the large-scale demonstration project of the Soil Erosion Service in adjacent Santa Cruz County. To what extent this has been true is impossible to say. Unquestionably our work had considerable effect which it would be hard to measure quantitatively. In one case a survey crew had almost completed the preliminary interviewing and took the supervisor to the farm to make final arrangements for doing a piece of control work. Upon reaching the site we were much surprised to find the work well under way along the lines suggested by the survey crew the day before. The owner had not waited for help from the S.E.R.A. crew. Again, an inexperienced farm operator seemed to welcome our work not merely because of the structures we installed on his property, but because of its value to him as a pattern for further work on his ranch. Yet another owner, on his own initiative, installed a structure supplementary to the S.E.R.A. work, before we had finished with his property.

#### SUMMARY

With a crew of S.E.R.A. workers a survey was made of erosion conditions in one supervisorial district of Monterey County, California, and experimental control measures were undertaken.

The results of the survey (which covered one-third of the area of the district) afford little basis for correlating erosion with soil, slope, or cover conditions, except that with a timber or brush cover erosion was inappreciable. On more than half the farms visited, erosion was noted, serious erosion on one-fifth of them. On one-fourth, some sort of control was practiced by the owner. On one-seventh, land use had been reduced because of erosion. Ill-advised farm management is responsible for much of the erosion observed.

The control measures included building of approximately 200 dams and other structures, largely of willow; planting willow and sea-fig; laying drain pipe; and digging diversion ditches. It was not possible within the limitations of an S.E.R.A. project to undertake a complete treatment of the erosion problem in the district, but the measures taken appear to be effective as far as they go.

# PHOSPHORUS CONTENT OF CITRUS AND FACTORS AFFECTING IT

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During an investigation of the phosphate nutrition of citrus, it was found that very few data were available regarding the phosphorus content of various portions of citrus trees. In order to supply this need and to study possible interrelations with other constituents, this factor was investigated; portions of the data obtained are here briefly described.

Total phosphorus in the dry matter was determined by the ashing method employed by Howk and DeTurk (11), followed by the molybdenum blue colorimetric method essentially as used by Truog and Meyer (14). The various phosphorus fractions in the dry matter were determined by the method of DeTurk, Holbert, and Howk (2).

## PHOSPHORUS IN CITRUS FRUITS

*Lemon fruits from Santa Barbara and Riverside.* During the spring of 1929, samples of Eureka lemons of various sizes were collected both at Santa Barbara and in field 3 at the Citrus Experiment Station; samples of Lisbon lemons were collected at Santa Barbara only. Data for the average fresh and dry weights per fruit (grams), the size class, the color, the percentage of phosphorus in the dry matter, and the average phosphorus content per fruit (grams) were obtained.

The flowers were found to contain the highest percentage of phosphorus of any stage in the fruiting process. The data obtained indicate the need of caution when dealing with tree-ripe fruits. If we do not consider these fruits, the percentage of phosphorus in the dry matter of lemons shows a decrease with increasing maturity of the fruit. In some cases the percentage of phosphorus in tree-ripe lemons shows an increase, and in some cases a decrease as compared with silver-stage fruit. Tree-ripe fruits from the different locations show approximately the same percentages of phosphorus in the dry matter. After maturity is reached, the fruits while attached to the tree apparently undergo changes, as regards phosphorus, that differ from those of the green stages. In addition, marked changes occur in the dry matter, the basis on which calculations are frequently made.

The average phosphorus content per fruit (grams) at various stages of growth is plotted in figure 1 against the average dry weight per fruit (grams). The graphs show that the content of phosphorus (grams) per average fruit becomes

greater with increasing maturity of the fruit and may be considered as a possible rough measure of fruit maturity. Gray (6) considers the effect of phosphates to be one of hastening the maturity of navel oranges. In the case of lemons from Riverside it is seen that after the fruits become a certain size, or have a given dry weight, the phosphorus content (grams) exceeds that of fruits from Santa Barbara. Since green fruits of a given size are most desirable in lemons, if the phosphorus content is a criterion of the degree of maturity,

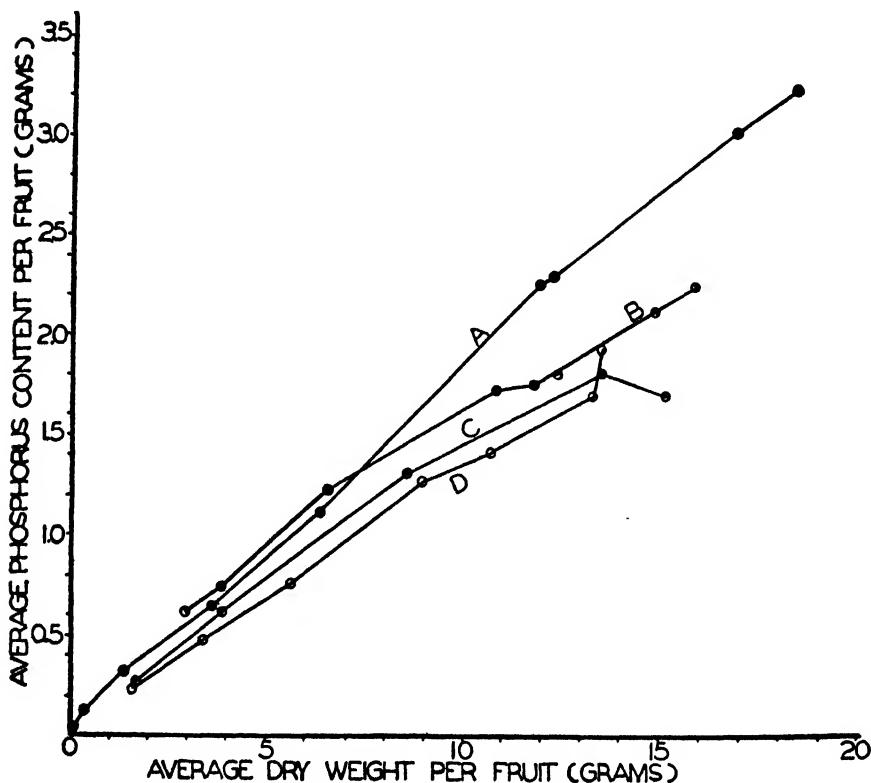


FIG. 1. RELATION OF AVERAGE DRY WEIGHT PER FRUIT (GRAMS) TO THE AVERAGE PHOSPHORUS CONTENT PER FRUIT (GRAMS) IN LEMON FRUITS

A, Eureka lemons collected April 16, 1929, at Riverside; B, Eureka lemons collected April 16, 1929, at Santa Barbara; C, Eureka lemons collected May 15, 1929, at Santa Barbara; and D, Lisbon lemons collected April 16, 1929, at Santa Barbara.

then Riverside fruits possibly do not remain in the green stage so long as do fruits at Santa Barbara. The increased phosphorus content of lemons at Riverside is of interest because of the prevalence of mottle-leaf in that citrus-growing area, and, as will be shown later, mottled leaves are uniformly richer in phosphorus than are healthy leaves grown under the same conditions.

A further point of interest in figure 1 is that Lisbon lemons of a given degree of size or dry weight contain less phosphorus than Eureka lemons grown under

the same environmental conditions. The growth of Lisbon lemon trees is usually more vigorous than that of Eureka lemon, and consequently a greater share of the phosphorus supply would be required for the increased vegetative growth, leaving less phosphorus available for the fruit. Furthermore, the active vegetative condition possibly would make a greater supply of organic materials available to the fruit and hence further dilute the phosphorus present.

TABLE 1  
*Phosphorus (P) content in fruits from trees in plots of Rubidoux fertilizer trials*  
(Per cent in dry matter)

PLOT	SOIL TREATMENT	VALENCIA ORANGES (PICKED MAY 15, 1929)		WASHINGTON NAVEL ORANGES (PICKED DEC. 19, 1929)		LEMONS (PICKED FEB. 25, 1930)	
		Peel	Pulp with seed	Peel	Pulp	Peel	Pulp with seed
A	Complete: Nitrate of soda, blood, bone, and sulfate of potash	.0725	.1938	.0688	.1531	.1900	.2600
B	No fertilizer	.0750	.1850	.0750	.2188	.1391	.2575
C	Dried blood	.0725	.1656	.0700	.1750	.1225	.2100
D	Sulfate of potash	.0738	.1900	.0988	.2500	.1425	.2350
E	Steamed bone	.0688	.1800	.0838	.2125	.....	.2438
F	Stable manure	.0600	.1656	.0713	.1750	.1325	.2375
G	Nitrate of soda, blood, and bone	.0600	.1725	.0650	.1719	.1338	.2150
H	Nitrate of soda	.1125	.2750	.0713	.1850	.2175	.2700
I	Muriate of potash	.0756	.1950	.0838	.2000	.1400	.2600
J	Superphosphate	.0775	.2100	.0813	.2150	.1450	.2563
K	Steamed bone and sulfate of potash	.0713	.1700	.0900	.2063	.1400	.2375
L	Nitrate of soda, blood, and sulfate of potash	.0688	.1863	.0663	.2000	.1375	.2188
M	No fertilizer	.0669	.1950	.0825	.2000	.1325	.2500
N	Superphosphate and blood to equal nitrogen in bone plots	.0700	.1900	.0650	.2000	.1125	.2500
O	Stable manure and rock phosphate	.0650	.1688	.0750	.1750	.1200	.2313
P	Steamed bone	.0600	.1563	.0900	.2000	.1375	.2400
Q	Complete: Nitrate of soda, blood, super- phosphate, and sulfate of potash	.0638	.1719	.0713	.1800	.1075	.2200
R	Sulfate of potash	.0700	.1850	.0788	.2000	.1275	.2500
S	Dried blood	.0588	.1500	.0750	.1800	.0963	.2125
T	Unfertilized	.0750	.2000	.0838	.2200	.1225	.2250
U	Manure, rock phosphate, and covercrop	.....	.....	.....	.2000	.....	.....
V	Manure, rock phosphate, and covercrop	.....	.....	.0750	.1875	..	.....

The phosphorus content of lemon fruits is a linear function of their dry weight; therefore the absolute amount of phosphorus (grams) continually increases in a fruit until it becomes mature. This phosphorus supply may be made available even to trees grown in soil solutions which contain relatively low phosphate concentrations. Burd and Martin (1) have demonstrated with water as the displacing agent that repeated displacement of the same mass of

soil with equal volumes of water results in a decreasing total concentration of electrolytes but usually in an increase in the phosphate concentration. The concentration of available phosphate therefore depends on the concentration of the other solutes and the reaction of their solutions.

*Phosphorus in peel and pulp of fruits from trees in plots of the Rubidoux fertilizer trials.* In 1929 samples of Valencia and Washington navel oranges, and in 1930 of lemon fruits, were collected from trees in the plots of the Rubidoux fertilizer trials. The peel was kept separate from the pulp. The data for the percentage of phosphorus (P) in the dry matter in relation to the soil fertilization are given in table 1. It is seen that the dry matter of the peel of lemons in general contains a higher percentage of phosphorus (average 0.1366) than that of the peel of oranges (average for Valencia orange peel 0.0709 and for Washington navel orange peel 0.0772). If averages are considered, the percentage of phosphorus in the dry matter of Valencia orange pulp was 0.1853, in Washington navel orange pulp 0.1958, and in lemon pulp 0.2390. The same general relation found in the peel holds also in the pulp. The peel or pulp of Valencia and Washington navel oranges showed no differences in percentages that could be related to the variety. Valencia orange and lemon peel or pulp from fruits of plot H that received nitrate of soda as its fertilizer treatment, contained markedly higher percentages of phosphorus than the peel or pulp of fruit from the Washington navel orange trees of that plot or of the other plots. The juice of Washington navel oranges from plot H was found to contain more inorganic phosphorus than that from the same variety of fruit from the other Rubidoux plots (8).

Examination of the trees of plot H in the rows in the field (15, p. 480) makes clear the following facts: From the irrigation standpipe at the head of the tree rows in plot H the trees are planted down the row in the order Washington navel orange, Valencia orange, Eureka lemon, and Lisbon lemon. The soil near the leaking standpipes received surface seepage water which did not extend beyond the Washington navel orange trees. Hence the navel orange trees were grown in a somewhat better-leached soil from which salts could move; the remaining trees in the rows were subject to the injurious physical and chemical effects of sodium salts on the soil. The effect of this more or less slow but continual leaching of the soil in which the navel orange trees were grown so improved the general appearance of these trees that it was almost impossible in 1929 to find a sufficient number of mottled leaves to serve as a sample. The beneficial effect of the slow leaching of the soil treated with sodium nitrate is evident from the fact that when Valencia orange trees were grown in soil in large tanks 8 feet in diameter by 4 feet deep and the soil was not permitted to become dry and was well drained, none of the injurious symptoms found in the Valencia orange or lemon trees of plot H were seen.

From a study of the pH of soil samples taken from plot H it is clear that if the first few drops of soil solution obtained by water displacement is tested, alkalinity to phenolphthalein may be obtained. Many of the plots of the Rubidoux trials far exceed the alkalinity of pH 7.6 without showing symptoms

of phosphate deficiency in the trees. Teakle (13) suggests that the hydroxyl ion alone probably causes the formation of a basic phosphate liberating some phosphate ions.

*Phosphorus in Valencia oranges grown in solution cultures and in the field.* Many of the leafy-twigg cuttings produced fruit, as can be seen in figure 1

TABLE 2

*Phosphorus in Valencia oranges grown on trees in phosphate-deficient or complete culture solutions, or on trees in the field at the Citrus Experiment Station*

VARIETY	GROWN IN	CONDITION AND PORTION OF FRUIT USED	PHOS- PHORUS IN DRY MATTER
			<i>per cent</i>
Valencia orange fruits from cutting grown on Lisbon lemon cutting as stock	Water culture (PO <sub>4</sub> absent)	1 fruit 3.5 cm. x 4 cm. long; 1 fruit 4 cm. x 4.5 cm. long; green; whole fruit	.0627
Valencia orange fruits from cutting	Water culture (PO <sub>4</sub> absent)	1 fruit 4.5 cm. x 4.5 cm. long; 1 fruit 4.5 cm. x 4 cm. long; green; whole fruit	.0560
Valencia orange fruit from cutting grown on Eureka lemon cutting as stock	Water culture (PO <sub>4</sub> absent)	1 fruit; full, ripe color; whole fruit	.0519
Valencia orange fruit from cutting grown on Lisbon lemon cutting as stock	Water culture (PO <sub>4</sub> absent)	1 fruit; full, ripe color; whole fruit	.0575
Valencia orange fruits from cutting grown on Rough lemon cutting as stock	Complete culture solution	1 fruit 6 cm. x 7 cm. long; 1 fruit 7 cm. x 7.5 cm. long; green; whole fruit	.2125
Valencia oranges from trees in plant pathology plot	Soil in field	5 fruits, mature; June 15, 1934; pulp (whole with seed); no peel	.2313
Valencia orange fruits plot I, R1, T29, Box Springs	Soil in field	22 young fruits, July 17, 1929; no buttons; fruits variable in size; whole fruit	.1959
Valencia orange fruits plot E, R1, T10, Box Springs	Soil in field	26 young fruits, July 17, 1929; no buttons; fruits variable in wise; whole fruit	.1959
Valencia orange fruits plot I, R45, T41, Box Springs	Soil in field	20 fruits, July 17, 1929; no buttons; fruits just shedding the style after the loss of petals; 2 to 2.5 cm. dia. x 2.5 to 3 cm. long; whole fruit	.1959
Valencia orange fruits block K, R45 (C)	Soil in field	35 young fruits, July 17, 1929; no buttons; fruits 2.5 cm. dia. x 3 cm. long; whole fruit	.2084

of plate 1. Some of these fruits were produced on cuttings grown in phosphate-deficient culture solutions, whereas others were produced by cuttings in complete culture solutions (control). For purposes of comparison, fruits of different ages were obtained from the field plots at the Citrus Experiment Station.

The results of the analyses for phosphorus are given in table 2. In the ab-

sence of phosphorus in the culture solution the percentage of phosphorus in the dry matter of Valencia oranges was between 0.0519 and 0.0575 for ripe fruit and slightly higher for smaller green fruit. When Valencia orange fruits were grown on cuttings in a complete culture solution containing 105 p.p.m. phosphate, the percentage of phosphorus in the dry matter was 0.2125, or approximately four times as high. Since many oranges grown on cuttings in culture solutions showed most severe granulation, the need for water is probably not a factor in the production of this physiological disease of Citrus fruits.

TABLE 3

*Phosphorus in the peel of citrus fruits grown on trees in phosphate-deficient or complete solution cultures or on trees in the field at the Citrus Experiment Station*

VARIETY	GROWN IN	CONDITION AND PORTION OF FRUIT USED	PHOSPHORUS (P) AS A PER CENT OF DRY MATTER IN			
			Stem half of peel		Tip half of peel	
			Inner portion	Outer portion	Inner portion	Outer portion
Valencia orange fruit from cutting grown on Eureka lemon cutting as stock	Water culture (PO <sub>4</sub> absent)	1 fruit; full ripe color; June 13, 1934	.0298	.0338	.0351	.0482
Valencia orange fruit from cutting grown on Lisbon lemon cutting as stock	Water culture (PO <sub>4</sub> absent)	1 fruit; full ripe color; June 13, 1934	.0191	.0233	.0223	.0283
Washington navel orange fruit from cutting grown on Rough lemon cutting as stock	Water culture (complete nutrient)	1 fruit, 6 cm. x 7 cm. long; 1 fruit, 7 cm. x 7.5 cm. long; green; June 13, 1934	.0483	.0659	.0529	.0889
Valencia orange fruit from trees in plant pathology plot	Soil in field	5 mature fruits; June 15, 1934	.0838	.1250	.1025	.1775

The dry matter of the whole pulp of Valencia oranges from the field contained 0.2313 per cent phosphorus. Four lots of the young oranges obtained in the field showed approximately 0.2 per cent of phosphorus in the dry matter of the whole fruit.

*Phosphorus in peel of oranges grown on trees in phosphate-deficient or complete culture solutions, or on trees in the field.* The stem and tip halves of the peel were divided into inner and outer portions. The latter included the oil glands. The data in table 3 show the distribution of phosphorus in the peel. The outer portion of the dry matter contains a greater percentage of phosphorus than the inner portion in both the stem and tip halves. The values for the tip halves exceed those for the stem halves. Phosphorus-free culture solutions permitted the growth of fruit but produced a marked reduction in the

percentage of phosphorus in the dry matter of the peel as compared with nearly ripe fruit grown on cuttings in complete culture solution.

The phosphorus content of citrus peel is of importance because of its possible relation to the oil of the peel. A sample of 10 cc. of commercial cold-pressed oil of orange showed 43 p.p.m. of phosphorus (if the specific gravity of the oil is assumed to be 1). The oil in the glands in the peel of Citrus fruits offers certain advantages to the fruit, such as protection; but at the same time there are disadvantages, such as occur when oil glands are injured and the oil itself injures otherwise healthy tissue. We invariably consider injury occurring to oil glands as being induced by mechanical abrasions, high temperatures, humidity, or water as shown by Fawcett, Klotz, and Haas (4). The possibility also exists that oils such as occur in Citrus peel contain certain fairly wide ranges of concentration of constituents such as phosphorus; when such concentrations are deficient or excessive, an instability may occur. Such oil composition possibly is a factor in the keeping quality of fruit under various

TABLE 4  
*Phosphorus fractions of Valencia orange peel*

	PORTION USED	PHOSPHORUS AS PER CENT OF DRY MATTER		
		Inorganic	Organic	Phospholipid
5 Valencia oranges, pathology plot, June 15, 1934	Stem half, inner portion	.080	.017	.014
	Stem half, outer portion	.093	.025	.015
	Tip half, inner portion	.085	.027	.016
	Tip half, outer portion	.128	.037	.016
7 Valencia oranges, Rancho Santa Fe, San Diego County, October 2, 1933	Stem half	.068	.015	.011
	Tip half	.076	.020	.012

packing-house and transportation conditions. The writer soon became aware of the fact that losses of oil, for example in drying samples of avocado fruits, occasion considerable losses in the phosphorus content. More information is needed regarding oil stability and its corresponding composition when fruits are grown under certain nutrient deficiencies. In addition to total phosphorus, data regarding the phosphorus fractions of Valencia orange peel are necessary for a more complete understanding of the phosphorus nutrition.

*Phosphorus fractions in Valencia orange peel.* The percentages of inorganic, organic, and phospholipid phosphorus in the dry matter of the peel of Valencia oranges obtained at Riverside and at Rancho Santa Fe were determined. The differences in inorganic and organic phosphorus occur in the same direction as that described for total phosphorus in table 3. The percentages of phospholipid phosphorus show the smallest differences, but these are in the same direction as those of the organic and inorganic phosphorus. The percentages of organic phosphorus slightly exceed those of phospholipid phosphorus and are considerably less than those of inorganic phosphorus (table 4).



Haas and Klotz (10) found a greater amount of oil in the peel of the stylar (tip) end of Valencia oranges than in that of the calyx (stem) end. As has just been discussed in connection with the possible relationship of phosphorus

TABLE 5  
*Phosphorus content of healthy citrus leaves*

VARIETY		DATE OF COLLECTION	LOCATION	PHOSPHORUS (P) IN DRY MATTER
Scion	Rootstock			
				<i>per cent</i>
Eureka lemon	Trifoliolate orange	Dec. 16, 1925	Rubidoux plots	.1150
Eureka lemon	Pomelo	.....	Rubidoux plots	.1050
Eureka lemon	Sweet orange	June 22, 1927	Rubidoux, plot B	.1056
Eureka lemon	.....	.....	Santa Barbara	.1425
Eureka lemon	.....	Aug. 23, 1927	Irvine	.0900
Eureka lemon	Pomelo	Jan. 25, 1928	Rubidoux, plot U	.1200
Eureka lemon	Sweet orange	Sept. 13, 1926	Rubidoux, plot T	.1625
Eureka lemon	Sour orange	Feb. 16, 1928	Rubidoux, plot U	.1100
Eureka lemon	Sweet orange	Feb. 16, 1928	Rubidoux, plot U	.1300
Lisbon lemon	Sweet orange	Oct. 14, 1927	Rubidoux, plot E	.1400
Lisbon lemon	Sweet orange	Aug. 17, 1927	Rubidoux, plot M	.1875
Lisbon lemon	Sweet orange	Aug. 17, 1927	Rubidoux, plot L	.0950
Lemon	Sweet orange	Aug. 17, 1927	Rubidoux, plot Q	.0950
Lemon	.....	.....	Santa Paula	.1374
Lemon	Sweet orange	.....	Rubidoux, plot N	.1375
Washington navel orange	Trifoliolate orange	Dec. 21, 1925	Rubidoux plots	.1244
Washington navel orange	Sweet orange	Dec. 16, 1925	Rubidoux plots	.1250
Washington navel orange	.....	July 19, 1928	Porterville	.1125
Washington navel orange	.....	Nov. 12, 1930	Alta Loma	.1250
Valencia orange	Trifoliolate orange	Dec. 16, 1925	Rubidoux plots	.1175
Valencia orange	Sweet orange	Dec. 31, 1925	Rubidoux plots	.1088
Valencia orange	Sour orange	Dec. 16, 1925	Rubidoux plots	.1238
Valencia orange	Pomelo	Dec. 21, 1925	Rubidoux plots	.1250
Valencia orange	.....	Oct. 28, 1930	Whittier	.0838
Valencia orange	.....	May 23, 1929	Escondido	.0750
Valencia orange	.....	April 14, 1929	Orange	.0838
Valencia orange	.....	May 22, 1929	Rancho Santa Fe	.0925
Valencia orange	.....	April 22, 1929	Tulare	.0913
Florida sour orange	Seedling	Aug. 5, 1927	Rubidoux plots	.1188
Mediterranean sweet orange	Seedling	.....	Rubidoux, plot U	.1350

to oil, the phosphorus fractions follow the same direction as that found by these investigators for the oil of Valencia orange peel.

*Healthy citrus leaves from trees grown in soil.* Although phosphorus is one of the chief constituents of a complete fertilizer, very few data are available

in regard to the phosphorus content of citrus leaves. Kelley and Cummins (12) presented some of the first reliable data in 1920 as analyses of controls in a study of mottled leaves, but their data are unfortunately most limited. More complete data are necessary, therefore, for a clearer conception of the range of the phosphorus content of leaves in the field and for later comparison with data obtained in artificial cultures under known conditions of phosphorus deficiency. Accordingly, the percentage of total phosphorus was determined in the dry matter of healthy citrus leaves of different varieties grown under widely different soil conditions and collected during a considerable period. The results are given in table 5. The dry matter of Eureka lemon leaves contained from 0.0900 to 0.1656 per cent of phosphorus, the lowest value being for leaves from trees at Irvine and the highest for leaves from trees on a check (no fertilizer) plot at the Rubidoux fertilizer trials. The results for phosphorus in Lisbon lemon leaves, which are confined to those for leaves of the trees on the Rubidoux plots, vary from 0.0950 to 0.1875 per cent in the dry matter. The phosphorus in Washington navel orange leaves varied from 0.1125 to 0.1250 per cent; that in Valencia orange varied from 0.0750 at Escondido to 0.1250 per cent at the Rubidoux plots. The values for Valencia orange leaves are uniformly higher for trees of the Rubidoux plots than for five other scattered locations. As we shall see in table 7, one may not conclude from the results shown in table 5 that the trees the leaves of which show the lowest values are deficient in phosphorus. Other factors must be taken into consideration.

*Effect of mottling and of soil fertilization.* At various times during 1930, healthy leaves and, whenever available, also mottled leaves were collected from the several groups of trees grown in the pathology plots at Riverside. Each of the various groups of trees represented a different combination of scion and stock. These trees were grown in soil that was given a uniform fertilizer treatment of a type used in profitable commercial practice. The percentage of phosphorus was determined in the dry matter of the leaf samples; and the results are shown in table 6.

In every case in which mottled leaves were available, they contained markedly higher percentages of phosphorus than did healthy leaves. Leaf samples collected at Hemet showed similar results. These findings confirm those of Kelley and Cummins (12). It is essential, therefore, in obtaining leaves for chemical analysis to avoid mottled leaves and to collect only healthy ones. The results in table 6 show no relation between the scion and stock and the percentage of total phosphorus.

The results thus far described regarding the phosphorus content of citrus leaves have dealt, first, with leaves from widely scattered locations in which the soil, fertilization, irrigation, climatic conditions, and other practices varied considerably; and secondly, with leaves from a uniformly treated grove in which the several varieties of trees were grown approximately under the same conditions. Consideration was next given a study of the relation of a single

practice such as fertilization to the phosphorus content of citrus leaves, with the full realization, however, that the fertilization of soil is not a simple process and that it may result in both physical and chemical changes in the soil with corresponding changes in other relations.

These leaf samples, which consisted of healthy and mottled leaves, were obtained in 1933 from Valencia orange trees grown on the various plots of the Rubidoux fertilizer trials. The healthy and the mottled leaves were collected from side by side positions, or close to one another on the same trees. All of the trees in plots A to T are budded on sweet orange rootstocks, and the trees chosen in plot V (rootstock plot) were also those on sweet orange.

TABLE 6

*Phosphorus content of gram aliquots of samples of citrus leaves obtained from mottled trees*

SCION	ORANGE STOCK	LEAF SAMPLES COLLECTED	LOCATION	PHOSPHORUS IN DRY MATTER OF LEAVES	
				Healthy	Mottled
				<i>per cent</i>	<i>per cent</i>
Valencia	.....	Jan. 14, 1931	Hemet	.1075	.1400
Valencia orange	Sweet	Oct. 3, 1930	Pathology plots, Riverside	.1425	.2200
Navel orange	Sweet	Oct. 3, 1930		.2000	.....
Navel orange	Sweet	Dec. 11, 1930		.1925	.2600
Navel orange	Sour	Oct. 3, 1930		.1600	.2375
Navel orange	Sour	Dec. 11, 1930		.2000	.....
Marsh grapefruit	Sweet	Oct. 3, 1930		.1525	.....
Marsh grapefruit	Sweet	Dec. 11, 1930		.1000	.1650
Marsh grapefruit	Sour	Dec. 11, 1930		.1025	.1450
Marsh grapefruit	Sour	Dec. 11, 1930		.1250	.....
Eureka lemon	Sweet	Oct. 3, 1930		.1300	.....
Eureka lemon	Sweet	Dec. 11, 1930		.1000	.1900
Eureka lemon	Sour	Oct. 3, 1930		.1213	.....
Eureka lemon	Sour	Oct. 3, 1930		.1125	.1563

The various plots received the different soil fertilizer treatments shown in table 7 and more fully discussed by Vaile (15). According to him the fertilization of the soil was started with relatively small amounts in 1907, the year the trees were planted. These amounts were gradually increased until 1914 when the plots received the following quantities annually until they were abandoned in July, 1933: 1.35 pounds actual nitrogen per tree; 2.70 pounds actual phosphoric acid per tree; and 1.35 pounds actual potash per tree. This means approximately 25 pounds per tree of 5-10-5 formula fertilizer (home-mixed) on the complete fertilizer plots, A and Q; 10 pounds of dried blood per tree on C and S; 9 pounds of nitrate of soda per tree on H; 14 pounds of steamed bone per tree on E, K, P; 2.5 pounds of sulfate of potash per tree on D, I, R; 13 pounds of superphosphate per tree on J; 8 cubic feet of manure per tree on V.

In table 7 the plots are arranged to correspond with the percentages of total phosphorus (P) in the healthy leaves. A study of the relation of the fertilizer

treatment to the percentages of phosphorus reveals the fact that the leaves of trees in plots V, C, Q, L, S, G, A, and H, which received the largest applications of nitrogen, contain the lowest percentages of phosphorus.

The data in table 7 show also the universality of the relation of higher percentages of phosphorus in mottled than in healthy leaves. Attention was directed in table 1 to the unusually high percentages of phosphorus in the

TABLE 7

*Phosphorus content of gram aliquots of Valencia orange leaf samples obtained from trees of the Rubidoux fertilizer trials*

PLOT	SOIL TREATMENT	LEAF SAMPLES COLLECTED (1933)	PHOSPHORUS IN DRY MATTER OF LEAVES	
			Healthy	Mottled
			<i>per cent</i>	<i>per cent</i>
V	Manure, rock phosphate, and cover crop	Feb. 18	.1025	.1375
C	Dried blood	Jan. 10	.1100	.1575
Q	Complete: nitrate of soda, blood, superphosphate, and sulfate of potash	Feb. 16	.1125	.1475
L	Nitrate of soda, blood, and sulfate of potash	Feb. 3	.1138	.1550
S	Dried blood	Feb. 17	.1150	.1325
G	Nitrate of soda, blood, and bone	Jan. 12	.1188	.1400
A	Complete: sulfate of potash, nitrate of soda, blood and bone	Jan. 9	.1200	.1800
H	Nitrate of soda	Jan. 12	.1400	.2000
P	Bone	Feb. 16	.1375	.1550
K	Steamed bone and sulfate of potash	Feb. 3	.1413	.2000
N	Superphosphate and blood to equal N of bone plots	Feb. 15	.1450	.2125
T	No fertilizer	Feb. 18	.1450	.2200
R	Sulfate of potash	Feb. 17	.1550	.2200
D	Sulfate of potash	Jan. 10	.1625	.1850
B	No fertilizer	Jan. 10	.1850	.2150
M	No fertilizer	Feb. 15	.1988	.2225
I	Muriate, at first; later, sulfate of potash	Jan. 12	.2100	.2500
J	Superphosphate	Jan. 11	.2150	.2600
E	Steamed bone	Jan. 11	.2150	.2500

Valencia orange and lemon fruits from trees of plot H. In table 7 the Valencia orange leaves from this plot contain the highest percentages of phosphorus in the group of high nitrogen-fertilized plots.

The percentages of phosphorus are next highest in the bone plots, with the single exception of plot E, and in plot N, which received nitrogen to equal that of the bone plots. The highest percentages of phosphorus occur in the group (plots T to J inclusive) which either received no fertilizer (control) or no nitro-

gen. The data, therefore, according to the nitrogen received by the plots, may be divided into three groups, the nitrogen fertilizer treatments of which roughly correspond inversely to the percentages of phosphorus in the leaves. High percentages of phosphorus in healthy leaves are indicative, therefore, of a relatively reduced nitrogen supply. In mottled leaves the percentages of phosphorus roughly fall into the same three groups as do the healthy leaves except that the percentages are all on a higher level.

The data in table 7 make it clear that a determination of phosphorus is not of itself an adequate basis at all times for conclusions regarding the needs of the leaves for phosphate. Fraps (5) and others have questioned the reliability of existing chemical methods of determining soil deficiencies in ash constituents. The data in table 7 indicate the caution necessary in drawing conclusions and especially when diseased leaves are to be taken into account. From these data one might conclude that the dry matter of healthy leaves of the best trees contains 0.10 to 0.14 per cent of phosphorus. The trees in plots G and H, however, were in general badly mottled and in poor condition, whereas the trees in plot V were in excellent condition. Consequently, 0.10 per cent or even somewhat less may not constitute a phosphorus deficiency.

The association of increased percentages of phosphorus with the mottling of citrus leaves does not necessarily indicate a causal connection between the two factors. The use of dilute phosphoric acid in large sand cultures with Valencia orange trees produced a chlorosis that approached, but was not, a true mottle. Unfortunately these sand cultures were in asphalt-coated galvanized iron containers which may have contributed zinc as an interfering factor. Haas (7) previously has reported the effect of adding dilute phosphoric acid to Sierra loam soil in 12-gallon earthenware pot cultures in which Valencia orange trees were grown, but in which drainage was permitted. Such cultures were repeated again out-of-doors. No drainage was allowed to take place, and applications of calcium nitrate were made intermittently. The phosphoric acid solution was reduced in strength to 1, 2, and 3 cc. in 18 liters of distilled water and was applied from time to time; otherwise, distilled water alone was used. At the two higher strengths several, but not all, of the trees showed mottle-leaf with the leaves frequently of reduced size. As the experiment continued, the mottled areas burned and the tissue in the yellow areas contracted. This is illustrated in figure 2 of plate 1. No mottling developed in leaves collected from similar but control trees grown in soil that was irrigated with distilled water and treated occasionally with calcium nitrate solution.

In October, 1934, samples of such affected Valencia orange leaves were collected from trees in soil that received 3 cc. of phosphoric acid in 18 liters of distilled water. Analysis showed that such leaves contained 1.175 per cent of phosphorus in the dry matter, which is the highest value yet found in citrus leaves. This total phosphorus content consisted of 0.718 per cent of inorganic, 0.255 per cent of organic, 0.039 per cent of phospholipid, and the remainder of acid-insoluble phosphorus. The association of higher percentages of phos-

phorus with mottling in citrus leaves is of interest further because of the results obtained by Eidelman (3), who found that the chlorophyll content of plants varies approximately inversely with the phosphorus content.

*Fractions in healthy and mottled leaves.* Samples of healthy and mottled leaves of several varieties of citrus were collected on December 11, 1930, from trees grown in the pathology plots at the Citrus Experiment Station. Healthy and mottled leaves of a given variety were collected from the same trees. In some cases the mottled leaves were adjacent to the healthy leaves and in others somewhat distant from them. Each bracket in table 8 represents samples from the same group of trees. The percentages of inorganic, organic, and phospholipid phosphorus in the dry matter were determined and the results are presented in table 8.

TABLE 8  
*Phosphorus fractions of healthy and mottled citrus leaves*

CONDITION OF LEAVES	SCION	ORANGE STOCK	PHOSPHORUS AS PER CENT OF DRY MATTER		
			Inorganic	Organic	Phospholipid
Healthy	Washington navel orange	Sweet	.125	.078	.018
Mottled	Washington navel orange	Sweet	.155	.096	.015
Healthy	Washington navel orange	Sweet	.138	.043	.017
Mottled	Washington navel orange	Sweet	.165	.057	.016
Healthy	Valencia orange	Sweet	.121	.065	.010
Mottled	Valencia orange	Sweet	.142	.078	.016
Healthy	Eureka lemon	Sweet	.095	.030	.009
Mottled	Eureka lemon	Sweet	.131	.036	.013
Healthy	Marsh grapefruit	Sour	.088	.039	.005
Healthy	Marsh grapefruit	Sour	.085	.022	.008
Mottled	Marsh grapefruit	Sour	.113	.035	.009

The percentages of inorganic phosphorus exceed in every case the sum of those of the organic and phospholipid fractions. Larger percentages of inorganic and organic phosphorus are clearly shown to occur in mottled leaves. With the exception of the percentages for Washington navel orange the percentages of phospholipids also were higher in the mottled leaves. The large preponderance of phosphorus in the inorganic form in citrus leaves makes its availability the greater to tissues in need of this element. The relatively low percentages of organic phosphorus are of interest because, in studies to be reported (9), it was found that there is a poor utilization of organic phosphorus by citrus unless the inorganic phosphorus supply is very low and that the phosphorus absorbed by citrus is largely in the inorganic form. It is desirable to know whether there are large differences in the total phosphorus content,

not only in the leaves and fruit, but also in the bark of trees in the field. Data in this regard do not exist. In studies of phosphorus deficiency it is essential that such basic data for healthy trees be available. Certain trees in the field at the Citrus Experiment Station which were about to be removed were used for such a study.

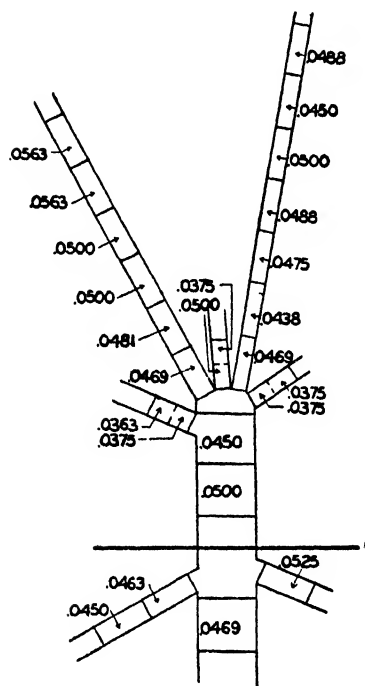


FIG. 2

FIG. 2. DIAGRAMMATIC REPRESENTATION OF THE PHOSPHORUS CONTENT (PER CENT IN DRY MATTER) OF 1-FOOT LENGTHS OF BARK OBTAINED FROM A VILLAFRANCA LEMON TREE ON SOUR ORANGE ROOTSTOCK GROWN IN THE FIELD AT THE CITRUS EXPERIMENT STATION

The tree was planted in June, 1917, and the circles of bark were cut on October 31, 1928. The heavy horizontal line designates the surface of the soil.

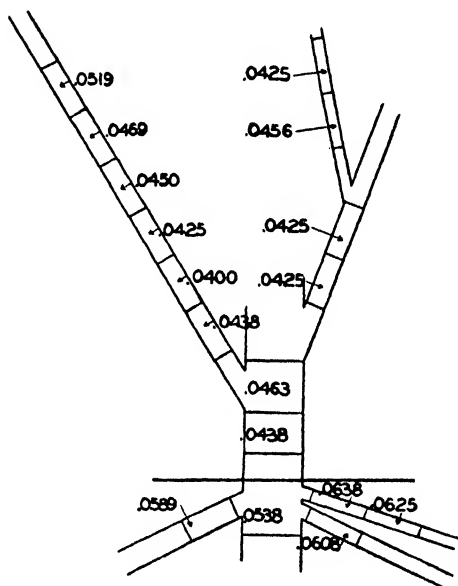


FIG. 3

FIG. 3. DIAGRAMMATIC REPRESENTATION OF THE PHOSPHORUS CONTENT (PER CENT IN DRY MATTER) OF 1-FOOT LENGTHS OF BARK OBTAINED FROM A LUE GIM GONG ORANGE TREE ON SOUR ORANGE ROOTSTOCK GROWN IN THE FIELD AT THE CITRUS EXPERIMENT STATION

The tree was planted in 1919, and the circles of bark were cut on October 31, 1928. The heavy horizontal line designates the surface of the soil.

*Phosphorus in citrus bark.* Determinations were made of the percentages of total phosphorus in the dry matter of the bark of Villafranca lemon trees. The results are diagrammatically represented in figure 2. The large 6-foot branch to the left in the figure shows values which increase slightly toward the tip;

the values for the sections from the 7-foot branch to the right show no definite trend, nor do the percentages for the trunk or rootstock. The several short branches show some of the smallest values. In general there is a fair degree of uniformity in the percentages of phosphorus in the bark of the trunk and largest branches and that of the large divisions of the rootstock.

Bark was also obtained from a Lue Gim Gong orange tree on sour orange rootstock. The percentages of phosphorus in the various 1-foot wide circles of bark are diagrammatically represented in figure 3. The values for the rootstock bark exceed those of the trunk and branches of the top. The 6-foot branch to the left in the figure shows a slight increase in the percentages in a direction toward the tip of the branch. These results show the relatively uniform distribution of phosphorus throughout the bark of the tree. This may be considered as indicative of the saturation of these tissues with phosphorus and of the storage of any excess in the leaves and youngest roots.

Bark samples were also collected from the trunks of orange trees grown on the plots of the Rubidoux fertilizer trials. Observation was made of the condition of the trees as regards mottle-leaf and of the manner of cleaning the bark prior to the removal of the bark samples. The samples were taken from the trunks about 6 inches above the bud union. The percentages of total phosphorus in the dry matter of the bark are remarkably uniform (0.0356 to 0.0463) regardless of the treatment of the samples during their collection. The bark of mottled trees showed no increased percentages of phosphorus, nor was there a relation of the percentages to the soil fertilizer treatment. The data suggest that the bark tissue is saturated with phosphorus.

In another study the percentages of total phosphorus were determined in the dry matter of the bark of sour orange rootstocks of young nursery trees that were budded to Washington navel orange.

One lot of trees was badly mottled. One group of 17 roots and another of 16 roots of the mottled trees were brushed free of soil while dry. The percentages of phosphorus found in the dry matter of this bark were 0.0750 and 0.0875, respectively. In another group of 15 roots of this same lot the rootstocks were first washed with tap water to remove adhering soil and then with distilled water. The percentage of phosphorus in the bark of this group was 0.0858.

A second lot of similar trees was obtained in which no mottling was evident. The bark of one group of 17 rootstocks that were brushed while dry contained 0.0829 per cent of phosphorus and that of another group of 16 rootstocks that were washed with tap water and then with distilled water contained 0.0900 per cent.

In these samples obviously the finest rootlets were not used because they could not be thoroughly cleaned and because the bark could not be separated. The data for the bark of these rootstocks showed a uniformity in the percentages of phosphorus, as did the bark from the trunks of older trees.

A study of the bark of the trunk of 3-year-old Rough lemon seedlings grown in soil showed some variations in the phosphorus content of the dry matter.



The bark of the shoots contained 0.0800 to 0.0825 per cent; that of the trunk: last elongation, 0.1200 per cent, upper half of remainder of trunk, 0.1100 per cent, lower half, 0.0925 per cent; that of main tap roots, 0.1175 per cent; and that of large lateral roots, 0.1063 per cent.

In January, 1927, analyses were made of the phosphate in the sap obtained by air suction from branches of Washington navel orange trees. The ash showed for mottled trees 41 p.p.m. and for healthy trees 43 p.p.m. phosphate, again revealing a uniformity.

#### SUMMARY

The phosphorus content and its relation to other constituents of various portions of citrus trees were investigated.

Lemon flowers contain the highest percentage of phosphorus of any stage in the fruiting process. The percentage of phosphorus in the dry matter of lemons (not tree-ripe fruit) shows a decrease with increasing maturity of the fruit. The content of phosphorus (grams) per average lemon fruit becomes greater with increasing maturity of the fruit. After the lemon fruits become a certain size or have a given dry weight the phosphorus content (grams) of the lemons from Riverside exceeds that of fruits from Santa Barbara. Lisbon lemons of a given degree of size or dry weight contain less phosphorus than Eureka lemons grown under the same environmental conditions.

The dry matter of the peel of lemons contains a higher percentage of phosphorus (average 0.1366) than that of the peel of oranges (average for Valencia orange peel 0.0709 and for Washington navel orange peel 0.0772).

If averages are considered, the percentage of phosphorus in the dry matter of Valencia orange pulp was 0.1853, in Washington navel orange pulp 0.1958, and in lemon pulp 0.2390. The same general relation found in the peel holds also in the pulp.

Valencia orange and lemon peel or pulp from fruits of plot H, which received nitrate of soda as its fertilizer treatment, contained markedly higher percentages of phosphorus than the peel or pulp of fruit from any trees of the other plots.

In the absence of phosphorus in the culture solution the percentages of that element in the dry matter of Valencia oranges were between 0.0519 and 0.0575 for ripe fruit and slightly higher for smaller green fruit; when the culture solution contained 105 p.p.m. phosphate, the percentage was 0.2125.

The outer portion of the dry matter of the peel of oranges contains a greater percentage of phosphorus than the inner portion in both the stem and tip halves. The values for the tip halves exceed those for the stem halves.

Phosphorus-free culture solutions permitted the growth of orange fruits but produced a marked reduction in the percentage of phosphorus in the dry matter of the peel as compared with nearly ripe fruit grown on cuttings in complete culture solution.

The phosphorus fractions in Valencia orange peel follow the same direction as was found for the oil of Valencia orange peel.

Analysis of healthy citrus leaves from trees in the field showed the dry matter of Eureka lemon leaves to contain 0.0900 to 0.1656 per cent of phosphorus; Lisbon lemon leaves, 0.0950 to 0.1875 per cent; Washington navel orange leaves, 0.1125 to 0.1250 per cent; and Valencia orange leaves, 0.0750 to 0.1250 per cent.

Mottled citrus leaves in every case contained markedly higher percentages of phosphorus than healthy leaves.

Healthy leaves of trees in plots that received the largest applications of nitrogen contain the lowest percentages of phosphorus. In healthy leaves high percentages of phosphorus are indicative of a relatively reduced nitrogen supply. The percentages of phosphorus in mottled leaves roughly fall into the same groups as do those in the healthy leaves, except that they are all on a higher level. Determinations of phosphorus in leaves are not of themselves adequate bases at all times for conclusions regarding the needs of the leaves for phosphate.

The percentages of inorganic phosphorus in citrus leaves exceed in every case the sum of those of the organic and phospholipid fractions.

There is a fair degree of uniformity in the percentages of phosphorus in the bark of the trunk and largest branches of a Villafranca lemon tree and in that of the larger subdivisions of the rootstock (sour orange). Data obtained in regard to the phosphorus content of healthy and mottled trees of plots of the Rubidoux fertilizer trials also show a marked uniformity and suggest that there is a saturation of the bark tissue of these mature trees with respect to phosphorus.

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#### PLATE 1

FIG. 1. Healthy growth of citrus cutting in culture solution without aeration in 21-quart shallow Swedish enamel ware pan. Valencia orange leafy-twigg cutting as scion on Rough lemon leafy-twigg cutting as stock. Culture solution contained 105 p.p.m. of phosphate for only a few days at intervals of several weeks. When phosphate was absent, 5 p.p.m. of aluminum was used. Iron, boron, and manganese are the only traces of elements added to the regular culture solution. Zinc was not added. Note the presence of a cluster of five large oranges and the absence of any mottling.

FIG. 2. Phosphate in relation to mottle-leaf. Valencia orange leaves from trees in Sierra loam soil cultures in 12-gallon earthenware pots without drainage. Calcium nitrate solution was occasionally added to permit healthy growth, as shown by the leaf at the left in the upper row. One to three cubic centimeters of phosphoric acid, diluted with distilled water to 18 liters, was occasionally added to certain cultures. In several of such cultures mottle-leaf developed, and as the leaves became fully mature the mottled areas began to dry out or burn.



FIG. 2



FIG. 1



# HYDRATION OF MINERALS AND SOIL COLLOIDS IN RELATION TO CRYSTAL STRUCTURE<sup>1</sup>

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Investigators have classified soil water as hygroscopic, water of hydration, adsorbed water, osmotic water, water of swelling, imbibed water, and various other types. Of course these terms are not meant to indicate that there are various kinds of water such as "heavy water" and "light water"; they are merely expressions of different relations that exist between the solid and the liquid phase. Strictly speaking, the numerous categories of soil water are vague designations or descriptions of the various forces which come into play in the process of water adsorption or hydration.

For a better understanding of soil-water relations in general, it becomes necessary to learn more about the specific surface forces which are characteristic of solids and which are able to attract water molecules. Since the present study is a first attempt in this direction, some of the conclusions are necessarily of tentative nature.

The colloids represent one of the most effective constituents of soils for water adsorption. Unfortunately, relatively little is known of these important substances, particularly in respect to the nature of their surfaces. It would appear to be hopeless to speculate about the absorptive forces of unknown surfaces, but the following mode of approach seems to be promising. By investigating the water relations of minerals of known composition and crystal structure and comparing the results with those obtained with soil colloids, one might then be able to interpret the hydration of the colloidal particles by way of analogy.

## SIGNIFICANCE OF VAPOR PRESSURE AND TEMPERATURE

If a dry surface is exposed to an atmosphere containing water molecules, the latter will strike the surface at random. If the surface contains attractive forces, some of the water molecules will be held and temporarily may stick to the surface. The greater the number of water molecules in the gas space, that is, the higher the vapor pressure, the more probable this mode of absorption will be. Evidently a strict control of the vapor pressure is essential in a study of water adsorption from the gaseous state.

<sup>1</sup> Paper No. 337, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

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Because of thermal agitation, the absorbed water molecules gradually escape again from the surface; for a given vapor pressure, this reverse process is the more pronounced the higher the temperature. Thus, temperature is another important variable in hydration studies.

In the present study, the vapor pressure was kept constant while the temperature was varied from 25° to 800°C. A relatively low vapor pressure (50 per cent  $\text{H}_2\text{SO}_4$ ) was selected in order to avoid ordinary water condensation, which would obscure the rôle played by the true surface forces of the solid.

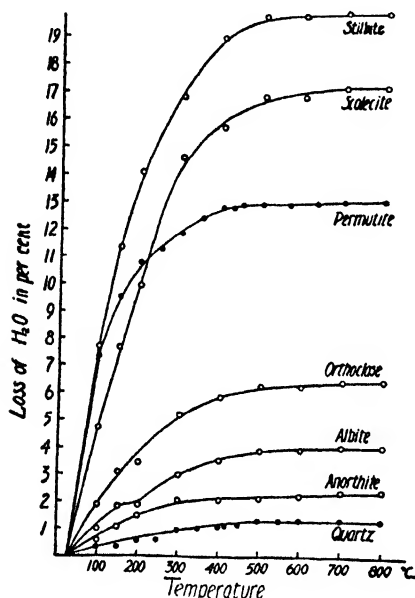


FIG. 1

FIG. 1. DEHYDRATION CURVES OF MINERALS OF THE LINKED TETRAHEDRA TYPE (FELSPAR TYPE)

In the case of quartz and feldspars the position of the flat branch of the curve is not a characteristic of the mineral but depends on the degree of fineness.

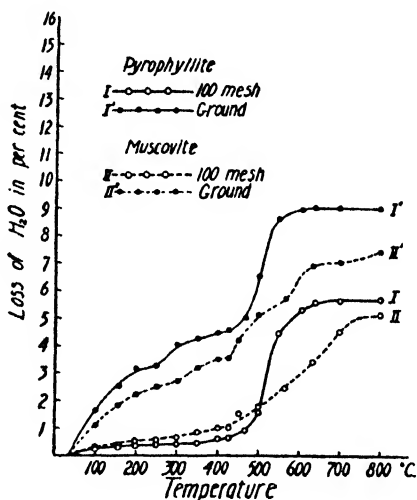


FIG. 2

FIG. 2. DEHYDRATION CURVES OF PYROPHYLLITE AND MUSCOVITE

In the 100-mesh samples the vertical branch (at 500°C.), which indicates crystal lattice water, is very pronounced.

#### EXPERIMENTAL METHOD

The materials used in this investigation were first ground as indicated herein-after and then brought to equilibrium with the atmosphere above 50 per cent  $\text{H}_2\text{SO}_4$  at a constant temperature of 25°C. The dehydrations were carried out against a similar atmosphere which was maintained by passing a current of air first through a series of towers containing 50 per cent  $\text{H}_2\text{SO}_4$  and then through the electric oven. The temperature was controlled by means of a thermocouple placed in close proximity to the crucibles in which the samples were held.

Approximately 1-gm. samples were used in duplicate and were kept at each temperature until loss in weight ceased.

#### DEHYDRATION CURVES OF SELECTED MINERALS

Figure 1 shows a family of curves, the members of which are nearly alike in shape. This group includes *quartz*, several *felspars*, two *zeolites*, and a *permutite*. Should the curve for a good-sized felspar crystal be plotted, it would run so close to the X-axis that it could not be shown on the scale chosen. The

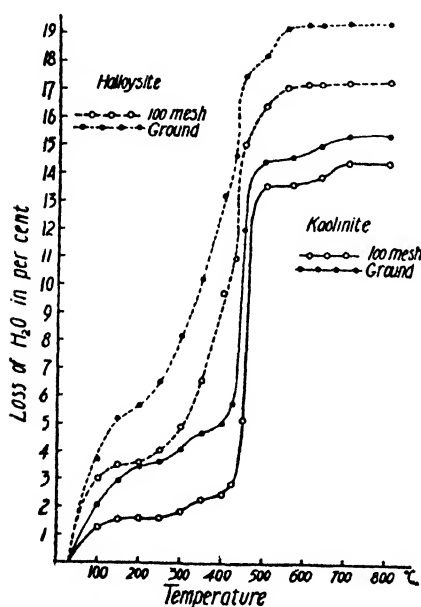


FIG. 3

FIG. 3. DEHYDRATION CURVES OF KAOLINITE AND HALLOYSITE

In the case of kaolinite note the extended vertical branch which corresponds to the water of crystallization.

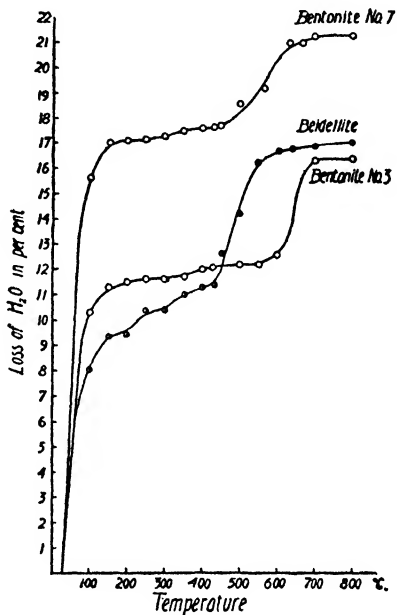


FIG. 4

FIG. 4. DEHYDRATION CURVES OF BEIDELLITE AND BENTONITES

The curves suggest the presence of adsorbed, as well as crystal lattice, water.

increased surface area caused by grinding the crystal shifts the dehydration curve upwards in proportion to the intensity of grinding. The fact that grinding increases the surface area can also be ascertained by measuring the increase in base-exchange capacity and the size of the particles (with sedimentation methods and the ultra-microscope). The end point (flat branch of curve) for all these curves is directly proportional to the base-exchange capacity. This clearly indicates that an increase in surface proportionally augments the water content of the substances; this water must have come from the atmosphere to which the minerals were exposed. Most of the water is given off below 400°C.



We shall designate this type of water as *adsorbed water*, or more specifically, *felspar water*. The relative rate of loss of the adsorbed water is the same for all the substances, which indicates that the same kind of adsorption forces are active on their exposed surfaces.

A different type of dehydration curve is given by the *micas* and *pyrophyllite* (fig. 2). The curves ascend gradually, and at the higher temperatures (above 500°C.) a marked loss of water occurs within a relatively narrow temperature interval. This pronounced change is commonly explained as escape of *crystal lattice water*.

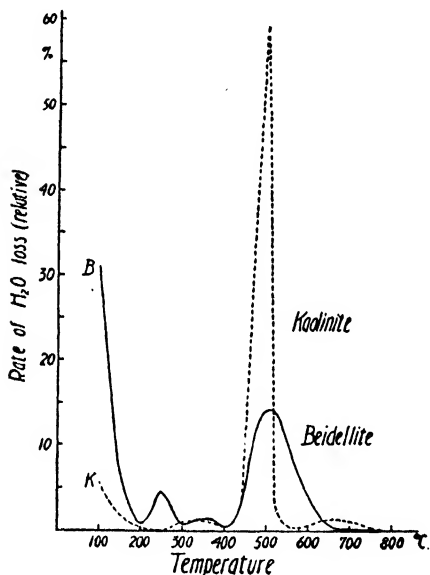


FIG. 5

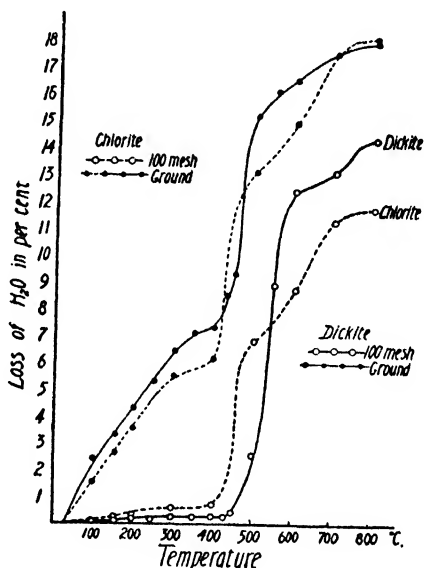


FIG. 6

FIG. 5. GRAPHS SHOWING THE EXPLOSION-LIKE LOSS OF CRYSTAL LATTICE WATER  
Relative losses per 50°C. intervals

FIG. 6. DEHYDRATION CURVES OF DICKITE AND CHLORITE

As in previous graphs, grinding increases the total water content but reduces the length of the vertical branch of the curve.

*Kaolinite* also exhibits in the curve a pronounced break, even more striking than that of *muscovite* (fig. 3). The inflection point occurs at a lower temperature, and the amount of the vertical shift in the curve is about twice as great as in *mica*, indicating a greater amount of water of crystallization. It is interesting to note that the dehydration curves for both *kaolinite* and *halloysite* agree well with those published by Ross and Kerr (10, 11).

An entirely different curve is given by the *bentonites* and *beidellite* (fig. 4). In a certain way the curves of these clays can be considered as combinations of types already discussed. There are, first, a pronounced water loss at moder-

ately low temperatures, which suggests the presence of adsorbed water, and second, an inflection point above 400°C., which is a strong indication of the presence of crystal lattice water. These curves will be of special significance in the study of soil colloids.

The curves presented in figure 5 merely represent a different way of plotting the data of kaolinite and beidellite previously shown. The lines show relative rates of water loss. The percentage of the total water lost for each increase in temperature of 50°C. was plotted against temperature. This mode of illustration brings out forcefully the explosion-like loss of crystal lattice water. According to modern concepts of crystal structure, the water molecules of these substances exist as OH ions and constitute an essential part of the lattice. High temperatures increase the thermal agitation to such a degree that the water molecules virtually jump out of the lattice with a consequent destruction

TABLE 1

*Differentiation of total water content of minerals into adsorbed and crystal lattice water (anhydrous basis)*

MINERAL	TOTAL WATER	ADSORBED WATER	CRYSTAL LATTICE WATER
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Muscovite . . . . .	5.37	1.04	4.33
Pyrophyllite . . . . .	6.00	0.64	5.36
Dickite . . . . .	16.65	0.49	16.16
Kaolinite . . . . .	16.86	2.80	14.00
Ca-Bentonite No. 3 . . . . .	19.65	14.59	5.06
Ca-Beidellite . . . . .	20.57	13.59	6.98
Halloysite . . . . .	20.91	4.29	16.62
Ca-Bentonite No. 7 . . . . .	27.09	22.49	4.60

of the crystal. The shattering of the mineral and the possible rearrangement of the atoms have been verified by X-ray methods.

Table 1 summarizes the content of crystal lattice water and adsorbed water in various minerals. It should be noted that the common minerals contain more lattice water than adsorbed water, whereas the bentonitic substances contain a much greater proportion of adsorbed water.

#### EFFECT OF GRINDING

Figure 6 shows the effect of increased specific surface on the hydration properties of *dickite* and *chlorite*. Dickite, ground to pass a 100-mesh sieve, yields a curve very similar to that of kaolinite (Ross and Kerr, 10). Upon further grinding, the total water content increases, as seen from the shift of the curve at 800°C. (14 → 18 per cent H<sub>2</sub>O). This is properly explained as an increase in adsorbed water due to greater specific surface area. It is of interest to note, however, that the extent of the vertical section has been changed; it appears

that the amount of crystal lattice water has been reduced. In addition, the position of the inflection point has been shifted to a lower temperature.

In figure 7, the data for *pyrophyllite* and *felspar* are plotted as relative percentages on an anhydrous basis. The coarse pyrophyllite loses about 90 per cent of its total water above 400°C., and the steep slope clearly indicates crystal lattice water. Grinding has produced two significant changes. First, the inflection point has been shifted to a lower temperature, indicating that crystal lattice water escapes more readily from the interior of small particles than from large ones. We are of the opinion that this influence of particle size constitutes an important feature in the interpretation of the dehydration curves of soil colloids. Differences in the inflection points do not necessarily signify different lattices but may be the result merely of variations in particle size.

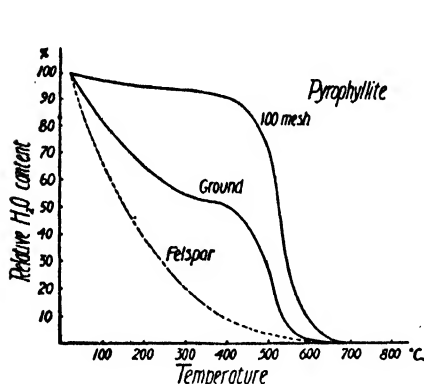


FIG. 7

FIG. 7. RELATIVE WATER CONTENT ON ANHYDROUS BASIS

Ground pyrophyllite occupies a position intermediate between coarse pyrophyllite and ground felspar.

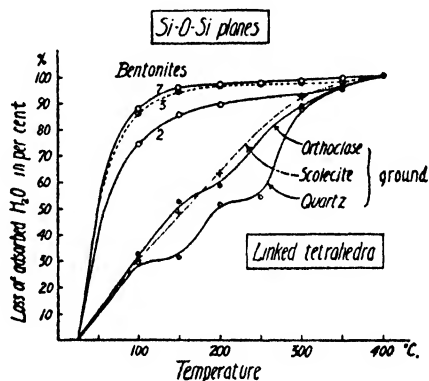


FIG. 8

FIG. 8. DIFFERENTIATION OF ADSORBED WATER INTO FELSPAR AND BENTONITIC TYPES OF ADSORBED WATER

Another result of grinding is the apparent reduction of crystal lattice water to 50 per cent as contrasted with 90 per cent before grinding. The difference appears as adsorbed water, that is, it is given off at lower temperatures. A plausible explanation is the following: In the interior of the lattice the OH ions are surrounded by other atoms or ions and the escape of H<sub>2</sub>O is difficult. If the OH ions are brought to the surface by way of grinding, they are held by the particle on one side only, they experience more freedom of movement, the extent of thermal agitation is greater, and they can escape at lower temperatures. In fact, it is very difficult to distinguish between adsorbed water and surface crystal lattice water in such instances. For comparison, the felspar curve has been plotted, and it is evident that further reduction of particle size would tend to bring the pyrophyllite curve even closer to the felspar line. The

important conclusion follows that in the range of colloidal dimensions the fundamental classification of minerals as anhydrous and hydrous loses some of its significance because the superficial crystal lattice OH ions act as adsorbed forms of water.

Table 2 gives a summary of the effect of increased specific surface. In all cases (except permutite), grinding increased the total water. In the case of hydrous minerals, grinding increased the amount of adsorbed water more than the total water, which was caused by crystal lattice water coming off below 400°C. Correspondingly, the amount of crystal lattice water, as found in coarser particles, was reduced. It should be remembered that we designate lattice water as that portion of total water which comes off at the beginning of

TABLE 2

*Changes in total adsorbed and crystal lattice water as a result of increased specific surface area (anhydrous basis)*

MINERAL	ADSORBED ION	INCREASE IN SATURATION CAPACITY	INCREASE IN TOTAL WATER	INCREASE IN ADSORBED WATER	DECREASE IN LATTICE WATER
		<i>m.e. per 100 gm.</i>	<i>gm. per 100 gm.</i>	<i>gm. per 100 gm.</i>	<i>gm. per 100 gm.</i>
<i>Hydrous minerals:</i>					
Muscovite.....	K	38	2.56	2.69	0.13
Dickite.....	H	39	5.12	8.45	3.33
Kaolinite.....	H	40	1.32	3.05	1.73
Bentonite No. 3.....	Ca, H	42	3.44	4.12	0.68
Pyrophyllite.....	H	80	3.94	4.28	0.34
Beidellite.....	Ca, H	151	0.75	2.19	1.44
<i>Anhydrous minerals:</i>					
Permutite.....	Ca	0	-0.03	-0.03	....
Quartz.....	H	3	0.62	0.62	....
Orthoclase.....	K	2	2.01	2.01	....
Biotite.....	K	53	3.92	3.92	....

the vertical branch of the curve, regardless of whether this amount is greater or smaller than the theoretical lattice water content of the ideal mineralogical formula.

#### PROPERTIES OF ADSORBED WATER

It seems desirable to discuss in greater detail the nature of the adsorbed water. For this purpose we have disregarded the lattice water and have set the total adsorbed water equal to 100. We shall call "adsorbed water" that proportion of total water which comes off at 400°C., provided there is no evidence that true crystal lattice water is included in this category.

In figure 8, the relative loss of adsorbed water has been plotted against temperature. Two groups of curves appear to be prominent: First, the feldspar, zeolite, and quartz lose their adsorbed water at a relatively uniform, but low

rate; secondly, the bentonites exhibit a strong bend in the curves, showing that the greatest fraction of their adsorbed water is lost at temperatures below 100° or 150°C. It seems that in general the adsorbed water is made up of different forms, and one might speak of *felspar* and *bentonitic types of adsorbed water*.

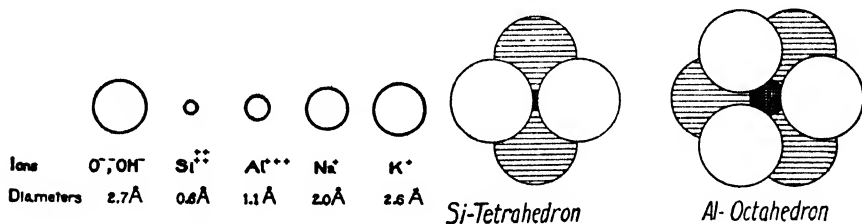


FIG. 9. SIZE OF IONS AS FOUND IN CRYSTALLIZED ALUMINO SILICATES

Si should be 0.8 Å instead of 0.6 as shown

FIG. 10. BUILDING STONES OF ALUMINO SILICATES

The large circles represent oxygen ions, the small ones silicon or aluminum ions.

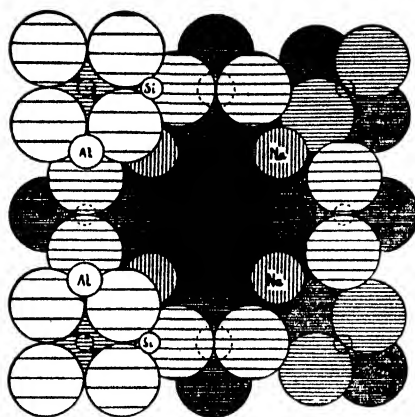


FIG. 11. STRUCTURE OF ULTRAMARINE

The large circles are oxygen ions [After Jaeger (5)]

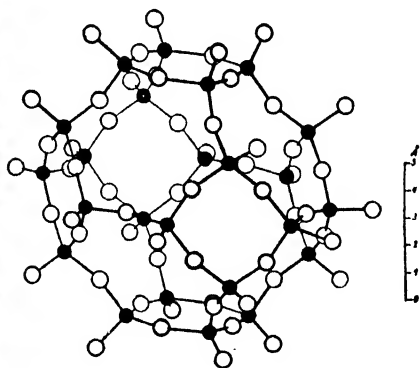


FIG. 12. SCHEMATIC NETWORK OF ULTRAMARINE

The broken bonds on the surface are shown [After Jaeger (5)]

The explanation is revealed by a study of the crystal structure of these substances. The one group (felspars, etc.) is made up of linked tetrahedra, whereas the bentonites are characterized by Si-O-Si planes. To explain these classifications fully it becomes necessary to discuss briefly modern concepts of crystal lattices.

## THE STRUCTURE OF ALUMINO SILICATES IN RELATION TO ADSORBED WATER

According to the viewpoint of Bragg (2), Goldschmidt (3), Pauling (8, 9), and others, aluminosilicates are made up of electrically charged particles which act as impenetrable spheres and which are properly called "ions." The *radii of the ions* are known (fig. 9). Attention is called to the important fact that O ions are the largest whereas Si and Al ions are so small that they can occupy the interstice in the center of four closely packed oxygen ions as shown in figure 10. Si or Al ions thus located are referred to as *silicon tetrahedra* or *aluminum tetrahedra*. In certain aluminosilicates the Al ion is surrounded by six O ions; this "radical" is called an *aluminum octahedron*. Si and Al tetrahedra and Al octahedra are the building stones of aluminosilicates, and the various structures are the results of variations in the arrangement of these ionic clusters.

*Definition of broken-bond water*

Figure 11 shows the arrangement, according to Jaeger (5), of tetrahedra in the case of *ultramarine*, a mineral related to the zeolites. The Al and Si ions are clearly recognizable. In the interior is seen a large cavity, which is the seat of the exchangeable ions (Na) and of loosely held water molecules (6). The same structure is shown in a more schematic manner in figure 12 in order to depict the three-dimensional linking of the tetrahedra. The black circles are Al or Si ions, the white circles indicate O ions. Every O ion is linked to two Si or Al ions. It is not possible to cut across the lattice without breaking the linkages, and it is clearly seen that the exposed ions possess a free "bond." We are of the opinion that the free bonds (Al, Si, O), which are the centers of strong electric fields, are responsible for the attraction of water molecules and thus constitute one type of adsorption force.

Water molecules are dipoles, that is, molecules in which the positive and negative charges are unsymmetrically arranged, and it is by virtue of this fact that they are strongly attracted by ions through the process of polarization and orientation. This type of linkage is relatively strong: considerable energy is necessary to disrupt it. We believe that such attraction is of major importance in the case of feldspars which are considered to have linked tetrahedra structures. Tentatively we shall designate the feldspar type of adsorbed water as "*broken-bond water*."

*Definition of planar water*

Other arrangements of tetrahedra are possible. Of special significance are the so-called "Si-O-Si planes" (oxygen planes), which are made up of rings consisting of six linked tetrahedra. Figure 13 shows such a plane as viewed from below. Theoretically, the area of such a sheet can be infinitely great. The most characteristic feature of an Si-O-Si plane, or of an OH-Al-OH plane, is the absence of unsaturated valences or free electric fields. In the plane itself there are no broken bonds and consequently no pronounced centers of

attraction. Nevertheless, the weak electric stray fields which are always present are able to attract water molecules. One would expect this form of adsorbed water to be loosely held and easily given off at low temperatures. We shall designate such adsorbed water as *planar water*.

Figure 14 shows the crystal structure of *kaolinite* and *montmorillonite* according to Hofmann, Endell, and Wilm (4). Note the existence of  $O^-$  and  $OH^-$  planes in these minerals. At the edges the planes are cut, and broken bond occur. The colloidal particles are built of a series of such plane packages.

Figure 15 gives a schematic picture of the *hydration conditions of a Si-O-Si plane*. The white circles denote O ions; the small black ones, Si ions within the tetrahedra. On the right, the plane is cut off, and free electric fields of O and Si ions attract water molecules (black circles), giving rise to broken-bond

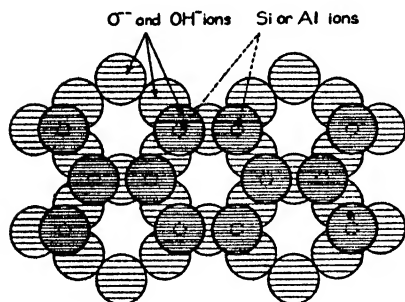


FIG. 13

FIG. 13. NEUTRAL O AND OH PLANE SEEN FROM BELOW

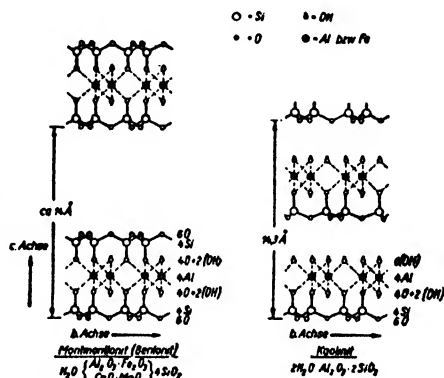


FIG. 14

FIG. 14. CRYSTAL STRUCTURE OF KAOLINITE AND MONTMORILLONITE

The lattice dimensions are correct, but the ion sizes are not drawn to scale. [After Hofmann, Endell, and Wilm (4).]

water. On the surface of the sheet we have the loosely attracted planar water (black circles). According to this viewpoint a substance consisting of planes has both broken bond and planar water, the relative proportion of the two depending on the shape of the particle. If the disintegration should take place mainly parallel to the planes, planar water would dominate; on the other hand, if grinding should break the particles chiefly across the planes, broken-bond water would exceed the planar water.

Table 3 presents a tabulation of broken-bond and planar water. The data indicate the percentages of adsorbed water given off at 150°C. All the materials referred to here were Ca-saturated at the outset in order to eliminate any possible variation due to the hydration effect of the adsorbed ion. The first two columns give the values for the feldspars, in which less than 50 per

cent of the total adsorbed water is lost at 150°C., indicating that the adsorbed water molecules are held tightly. These figures are characteristic of broken-

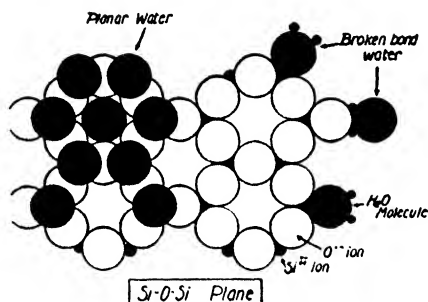


FIG. 15

FIG. 15. HYDRATION OF A NEUTRAL Si-O-Si PLANE

The white circles represent oxygen ions; the small black circles, silicon ions; and the large black circles, adsorbed water molecules.

FIG. 16. DEHYDRATION CURVES OF REDDING, CECIL, AND SIERRA SOIL COLLOIDS

The  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio is 2 or less

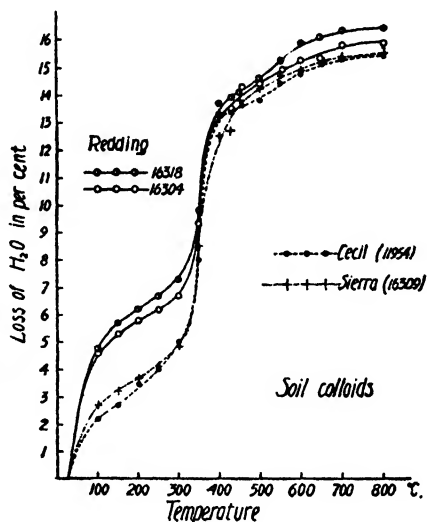


FIG. 16

TABLE 3

Per cent of adsorbed water lost at 150°C.

BROKEN-BOND WATER		PLANAR WATER		MIXTURES	
Mineral	H <sub>2</sub> O	Mineral	H <sub>2</sub> O	Mineral	H <sub>2</sub> O
	per cent		per cent		per cent
Albite II	34.2	Bentonite No. 8	85.4	Biotite	55.1
Labradorite	38.6	Bentonite No. 2	85.6	Muscovite	56.1
Anorthite	44.0	Bentonite No. 6	88.5	Pyrophyllite	61.2
Albite I	45.4	Bentonite No. 7	89.2	Kaolinite I	67.1
Orthoclase	47.8	Bentonite No. 3	90.3	Kaolinite II	47.8
Oligoclase	49.0	Beidellite	92.2	.....	.....
.....	.....	Bentonite No. 3a	93.0	.....	.....
.....	.....	Bentonite No. 7a	95.8	.....	.....

bond water. The middle columns represent planar water. It will be noted that in this case from 85 to 96 per cent of all adsorbed water leaves the surface at 150°C., a fact which points toward the dominance of very weak physical or



chemical bonds. Bentonite and beidellite are typical examples of this group of substances. The last set of columns comprises intermediate cases. Here the areas of the planes and the broken lattices are of similar magnitude and the hydration values fall between the two categories.

#### DISCUSSION OF SOIL COLLOIDS

The study of hydration and dehydration of minerals of known structure has revealed the following facts: First, it is possible to evaluate the amount of crystal lattice water and its critical temperature of escape; secondly, the amount of adsorbed water can be determined; and thirdly, the water of adsorp-

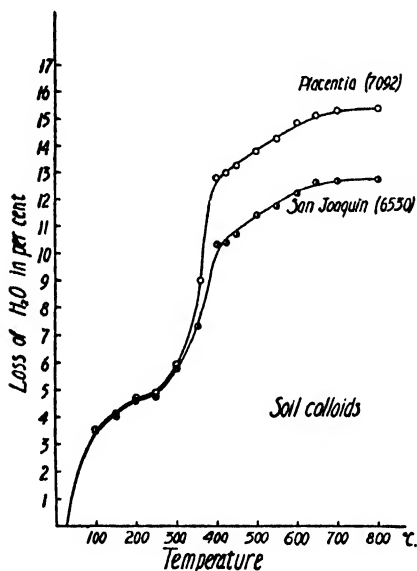


FIG. 17

FIG. 17. DEHYDRATION CURVES OF PLACENCIA AND SAN JOAQUIN SOIL COLLOIDS

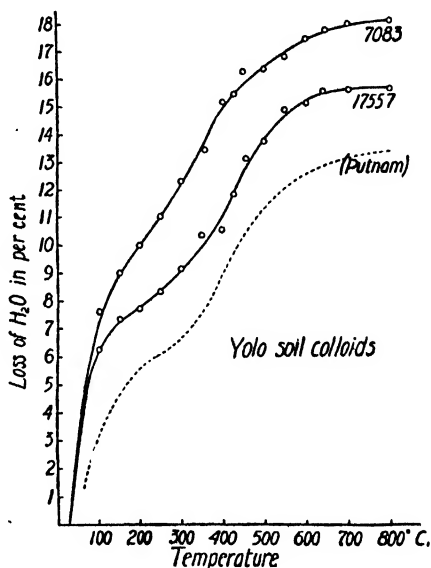


FIG. 18

FIG. 18. DEHYDRATION CURVES OF YOLO COLLOIDS

The  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio is approximately 3

tion can be divided into broken-bond and planar water. The question arises as to whether soil colloids exhibit similar relations. Accordingly, about 40 different soil colloids (1 micron or less) were saturated with Ca and then subjected to the same technique as the minerals already discussed. The results are typified by figures 16, 17, 18, and 19.

*Redding, Cecil, Sierra.* Figure 16 illustrates four soil colloids of reddish color and low  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios. The Cecil, from Alabama, and the Sierra, from California, were derived from acid igneous rocks and, though they were formed several thousand miles apart, they are practically alike in their dehydration properties. A steep vertical branch is evident, and suggests the pres-

ence of crystal lattice water. It should be noted that the critical temperature at which lattice water escapes is below 400°C.

*Placentia, San Joaquin.* Colloids were isolated from strongly weathered alluvial deposits in California (fig. 17). The two soil types are supposed to be genetically related, the San Joaquin being the more mature. In regard to adsorbed water the two colloids are identical, the main difference being shown by the length of the vertical branch of the crystal lattice water curve.

*Yolo colloids.* Of very different nature are the Yolo colloids of California, which appear to be related to the Putnam colloids of Missouri (1) (fig. 18). A smooth, wave-like shape is typical for these dehydration curves. The presence of crystal lattice water is indicated by the inflection point, but the escape

TABLE 4

*Adsorbed water and crystal lattice water in soil colloids (anhydrous basis)*

COLLOID	LABORATORY NUMBER	TOTAL WATER	ADSORBED WATER	CRYSTAL LATTICE WATER
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Cecil.....	11954	18.04	4.74	13.30
Sierra.....	16309	18.19	4.81	13.38
Redding.....	16318	19.40	8.01	11.39
Redding.....	16304	18.69	7.22	11.47
San Joaquin.....	6530	14.54	5.41	9.13
Placentia.....	7092	18.03	5.74	12.29
Yolo.....	7083	21.83	12.12	9.67
Yolo.....	17557	18.25	10.53	7.72
Susquehanna A.....	16312	19.12	10.61	8.51
Susquehanna B.....	16313	21.07	14.01	7.06
Susquehanna C (10-20 inches).....	17554	20.75	13.39	7.36
Susquehanna D (10 feet).....	17555	21.08	14.12	6.96
Susquehanna E (11 feet).....	17556	29.29	23.61	5.68
Putnam.....		15.30	7.25	8.05

of the lattice water appears to be less violent than in the case of the previous colloids.

Table 4 shows a series of soil colloids tabulated according to their crystal lattice water and adsorbed water. Generally speaking, the total water content of these colloids is high; this fact is undoubtedly related to the small particle size and consequent great specific surface. As in the case of the minerals, two groups can be recognized; namely, those colloids which have more crystal lattice water than adsorbed water (Cecil type), and those in which this relation is reversed (Yolo, Susquehanna).

*Susquehanna profile.* Of great interest are the curves of the Susquehanna colloids (fig. 19) which were extracted from a Susquehanna profile located in the southeastern United States (Alabama). The curve of the so-called E-horizon (11 feet in depth), stands apart from the others of this profile; a comparison

with the curves for the minerals clearly points toward a very close resemblance to bentonite. The remaining curves form a uniform family with regular alterations according to depth. The higher the base-exchange capacities, the steeper the initial slope of the curves. The presence of lattice water is beyond doubt, if analogies are at all justified.

#### NATURE OF SURFACE OF CLAY PARTICLES

Table 5 contains various data of considerable significance. In column 3 is shown the percentage of adsorbed water lost at 150°C.; in the majority of the cases the percentages are high, a fact which we interpret as indicative of the

TABLE 5

*The relation of planar water in soil colloids to base-exchange capacity (Ca-saturated colloids)*

COLLOID	LABORATORY NUMBER	ADSORBED H <sub>2</sub> O LOST AT 150°C.	BASE- EXCHANGE CAPACITY	H <sub>2</sub> O LOST AT 150°C. (MOIST BASIS)	RATIO OF TOTAL WATER LOSS AT 150°C. TO BASE- EXCHANGE CAPACITY	MOLECULES H <sub>2</sub> O PER EXCHANGE SPOT
1	2	3	4	5	6	7
		<i>per cent</i>	<i>m.e. per 100 gm.</i>	<i>gm. per 100 gm.</i>		
Cecil.....	11954	67.6	17	2.66	0.156	8.65
Sierra.....	16309	80.5	19	2.89	0.152	8.44
Redding.....	16318	84.7	35	5.38	0.154	8.55
Redding.....	16304	87.0	31	5.01	0.162	9.00
San Joaquin.....	6530	98.0	27	3.91	0.145	8.06
Placencia.....	7092	83.4	25	3.92	0.157	8.72
Yolo.....	7083	90.2	67	8.98	0.134	7.48
Yolo.....	17557	55.8	56	7.91	0.141	7.84
Susquehanna A.....	16312	59.4	30	4.73	0.158	8.78
Susquehanna B.....	16313	65.0	38	6.68	0.175	9.72
Susquehanna C.....	17554	78.2	60	7.84	0.131	7.28
Susquehanna D.....	17555	87.4	62	9.18	0.148	8.23
Susquehanna E.....	17556	92.0	95	14.18	0.149	8.28
Putnam.....	.....	67.6	60	....	.....	....

presence of planar water. Probably these colloids contain O<sup>2-</sup> or OH<sup>-</sup> planes, and furthermore, these planes appear to be of greater extent than the broken-bond areas, from which one would conclude that the particles are of platy shape. This conclusion is supported by optical data.

In column 6 of table 5, the 150°C. water (absolute basis) has been divided by the base-exchange capacity; a surprisingly constant ratio results. As shown in column 7, from 7 to 9 water molecules are associated with each exchange spot, or 14 to 18 per adsorbed Ca ion. These values are much too high to be explained by Ca-ion hydration alone. Other attraction spots for water must exist on the clay surface, namely, the broken bonds and the planes.

In figure 20 the exchange capacities are plotted against the water loss at

150°C. Two curves result. First we have the minerals consisting exclusively of linked tetrahedra with broken-bond water. Quartz, feldspars, and zeolites center around a common line. The second curve comprises the bentonite and soil colloids. It is much steeper than the lower curve because the number of water molecules per exchange spot is several times greater. The nearly linear relation in the case of the soil colloids strongly suggests that the soil-colloid surfaces are more like those of the bentonites and distinctly different from the

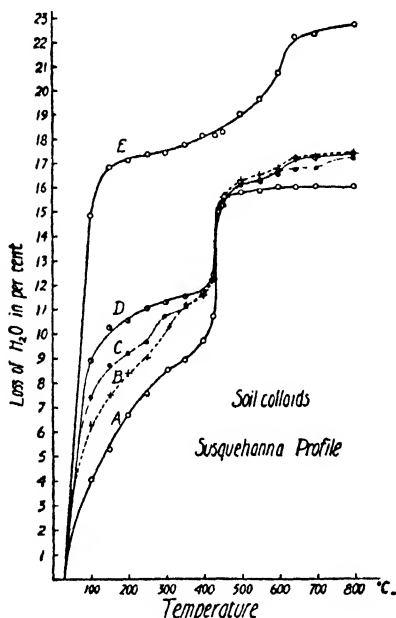


FIG. 19

FIG. 19. DEHYDRATION CURVES OF COLLOIDS EXTRACTED FROM THE SUSQUEHANNA PROFILE

Note the characteristic changes occurring with increasing depth (A → E). Curve E closely resembles that of Bentonite No. 7 (fig. 4).

FIG. 20. TOTAL WATER LOSS AT 150°C. AS A FUNCTION OF BASE-EXCHANGE CAPACITY

The soil colloids fall in line with the bentonitic colloids, which contain relatively much planar water, rather than with the feldspar-zeolite minerals, which contain broken-bond types of water.

linked tetrahedra type. One is inclined to consider this as additional evidence of the existence of  $O^{--}$  and  $OH^-$  planes in soil colloids.

#### SUMMARY OF SOIL COLLOID STUDIES

The following conclusions appear to be justified: Soil colloids contain water of crystallization or, more precisely speaking,  $OH^-$  ions, as parts of a crystal lattice structure. This may be considered as an independent proof of the

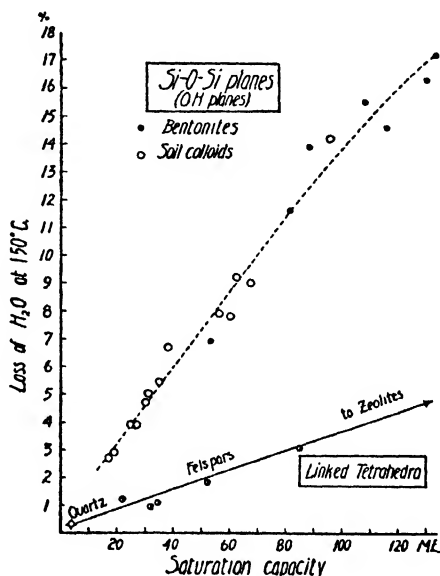


FIG. 20

crystalline nature of the colloidal particle (7). Unlike the minerals of known structure, the soil colloids lose their lattice water at lower temperatures. Whether this is caused merely by particle size or indicates a structural peculiarity, or both, must be further investigated. At least two major classes of soil colloids appear to exist: those which resemble in some measure kaolinite and halloysite; and those which appear to be related to (but not identical with) beidellite. In all cases, however, the composition of the surfaces seems to be rather similar; Si-O-Si planes and possible OH planes probably exist, and planar water dominates over broken-bond water.

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## SOIL SWELLING: II. SWELLING OF SOIL IN SOLUTIONS OF ELECTROLYTES; MICROSCOPIC AND X-RAY INVESTIGATIONS

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Various theories exist concerning the influence of neutral salts on swelling. The old theory that cations and anions of salts exert a specific action on swelling is based on the ideas of Hofmeister, who established ion sequences and their rôle in this process.

Loeb objects to the ion sequences of Hofmeister. He assumes that cations and anions do not exercise any specific influence on the phenomenon of swelling and that the influence of salts is determined by the active acid reaction of the medium (pH). Chlorides, bromides, nitrates, and other anions in the form of neutral salts do not play any rôle.

On the contrary, new researches have demonstrated that the action of acids on the swelling of proteins depends not only on the pH but to a considerable degree on the nature of the anion also (17). This was demonstrated with still more conclusive evidence by Meunier and Rey (15), who confirmed the applicability of the anion sequences to the swelling of wool in neutral salt solutions though at the moment of equilibrium the pH varied within a wide range (from 4.5 to 7.4).

Michaelis (16) explains the action of very dilute solutions of salts by the size and kind of the charge of their ions. No specific difference is observed in the action of different ions. At high dilutions the action of ions may be explained by the concentration of H ions (pH). On the contrary, in concentrated solution the influence of the specific action of the salt ions takes the first place.

The influence of salts on the swelling of soil colloids and of bentonite has been studied by Mattson (3, 14). He has shown that in accordance with Donnan's equilibrium swelling decreases as a result of the addition of free electrolytes. The higher the valence of the ion of the same sign as the colloid's charge the less is this decrease of swelling. The higher the valence of the ion of the sign opposite to that of the colloid's charge the greater is the decrease of swelling. Hofmeister's sequences are applicable to soils also. The influence of the cations is, however, not quite clear here.

Herzog (6) in his investigation of the swelling of cellulose thinks it possible to apply to the explanation of the phenomena of swelling in electrolyte solutions the notions developed by Fajans in regard to the hydrophillic nature of ions. The sequence of ions according to their hydrophillic nature is represented by him as follows:

Monovalent cations— $H > Li > Na > K > Rb > Cs > NH_4$

Divalent cations— $Mg > Ca > Sr > Ba$

Monovalent anions— $F > Cl > Br > J \sim NO_3 \sim ClO_3 \sim CNS > OH$

Divalent anions— $CO_3 > SO_4$ .

The cations hydrate more strongly than the anions. Swelling decreases with the decrease of the hydrophillic nature of cations. The swelling capacity expresses the sum total of the hydrophillic nature of the ions composing the salt.

An almost similar sequence of cations according to their influence on hydration of clay is presented by Bayer and Horner (1)



#### EXPERIMENTAL

The experiments on swelling in solutions of electrolytes were designed to regulate the process of swelling in soil by means of adding salts, e.g., calcium salts; to study the influence of salts on structure stability; and to observe simultaneously swelling and absorption.

TABLE 9  
*Swelling of columnar alkali soil in  $\text{CaCl}_2$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$	ABSORP- TION PER 100 GM.
							<i>m.e.</i>
Water.....	21.66 $\pm 0.69$	40.05 $\pm 0.7$	46.44	13.16	0.58	86.22	0
0.1 N.....	25.38	41.58	47.84	12.81	0.59	86.9	4.71
1.0 N.....	21.25	39.17	49.40	12.63	0.59	79.46	39.80
4.0 M.....	11.25	29.76	43.55	8.42	0.68	68.33	.....

TABLE 10  
*Swelling of loamy chernozem in  $\text{CaCl}_2$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$	ABSORP- TION PER 100 GM.
							<i>m.e.</i>
Water.....	15.30 $\pm 0.18$	42.36 $\pm 0.58$	55.53	8.71	0.76	76.13	0
0.1 N.....	14.32	40.65	54.30	7.66	0.78	74.82	0
1.0 N.....	12.76	39.80	54.49	7.58	0.78	73.06	0
4.0 M.....	6.15	32.40	50.0	3.23	0.89	64.80	.....

The influence of the cations Ca, Mg, Na, Ba, and H was studied. For the sake of comparison all the salts were taken in the form of chlorides; the only acid employed in these tests was  $\text{H}_2\text{SO}_4$ . The fact that the curve of swelling may express either stabilization or the coagulation of the sol in respect to the electrolyte (18) was taken into account. The method used for studying swelling in the solutions of electrolytes was described in our preceding work<sup>1</sup> (20).

<sup>1</sup> The following values are the same as in the preceding work (20).  $Q$  = maximum swelling in volume units.  $K_{\Delta}$  = the amount of solution absorbed in cc.  $P_{\Delta}$  = the changed total porosity of the swollen soil.  $W_q$  = "swelling water" = "swelling solution."  $S$  = structure coefficient.  $\frac{K_{\Delta}}{P_{\Delta}}$  = the capillary relative porosity.

TABLE 11  
*Swelling of columnar alkali soil in  $MgCl_2$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$
Water.....	21.66 $\pm 0.69$	40.05 $\pm 0.7$	46.44	13.16	0.58	86.22
0.1 N.....	20.45	40.0	47.03	13.60	0.57	85.05
0.5 N.....	21.09	40.19	47.23	13.49	0.57	85.10
1.0 N.....	19.17	36.15	46.45	10.90	0.63	77.82

TABLE 12  
*Swelling of loamy chernozem in  $MgCl_2$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$	ABSORP- TION PER 100 GM.
Water.....	15.30 $\pm 0.18$	42.36 $\pm 0.58$	55.53	8.71	0.76	76.13	<i>m.e.</i> 0
0.1 N.....	13.24	40.97	53.51	8.38	0.76	76.54	1.68
1.0 N.....	15.56	39.75	52.34	6.40	0.81	75.94	13.80

TABLE 13  
*Swelling of columnar alkali soil in  $BaCl_2$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$
Water.....	21.66 $\pm 0.69$	40.05 $\pm 0.7$	46.44	13.16	0.58	86.22
0.1 N.....	20.13	38.76	45.09	12.74	0.59	85.97
1.0 N.....	17.71	37.15	47.43	12.35	0.60	78.33

TABLE 14  
*Swelling of columnar alkali soil in  $NaCl$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$
Water.....	21.66 $\pm 0.69$	40.05 $\pm 0.7$	46.44	13.16	0.58	86.22
0.1 N.....	19.57	39.93	45.85	13.90	0.57	87.09
0.5 N.....	19.25	38.97	45.45	13.19	0.58	85.74
1.0 N.....	21.89	36.53	44.36	11.39	0.62	82.35



Tables 9-17 show the results of these experiments. In computing the value of  $K_{\Delta}$  the amount of solution absorbed was expressed in cubic centimeters, its specific volume being taken into account. This makes the values of  $K_{\Delta}$  comparable. As an indicator of swelling we use the quantity we call "swelling

TABLE 15  
*Swelling of columnar alkali soil in  $H_2SO_4$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$
Water.....	21.66 $\pm 0.69$	40.05 $\pm 0.7$	46.44	13.16	0.58	86.22
0.1 N.....	20.44	38.46	47.82	12.38	0.59	80.42
0.5 N.....	25.24	37.39	50.59	9.73	0.65	73.91

TABLE 16  
*Swelling of loamy chernozem in  $H_2SO_4$  solutions*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$	ABSORP- TION PER 100 GM.
Water.....	15.30 $\pm 0.18$	42.36 $\pm 0.58$	55.53	8.71	0.76	76.13	m.e. 0
0.1 N.....	14.48	42.04	54.29	8.79	0.75	77.43	6.86
0.5 N.....	19.06	44.30	56.25	8.69	0.76	78.75	14.12

TABLE 17  
*Swelling of loamy chernozem saturated with Na to the amount of 27.94 per cent of its exchange capacity*

CONCENTRATION OF SOLUTION (AVERAGE)	$Q$	$K_{\Delta}$	$P_{\Delta}$	$W_q$	$S$	$\frac{K_{\Delta}}{P_{\Delta}} \cdot 100$	ABSORP- TION PER 100 GM.
Water.....	17.68	41.58	55.86	9.48	0.74	74.43	m.e. 0
0.1 N $CaCl_2$ .....	18.10	35.41	51.95	2.42	0.90	68.16	2.29
1.0 N $CaCl_2$ .....	14.90	33.84	50.39	1.91	0.93	67.15	28.12

solution" ( $W_q$ ), analogous to the "swelling water" of our preceding work, instead of the habitually used "volume increase."

The results of the determinations are shown in figures 2 and 3.

With loamy chernozem no absorption of Ca from the 0.1 N and 1.0 N solutions of  $CaCl_2$  was observed. The swelling curves ( $W_q$ ) for the two soils have one maximum for distilled water, from which they sink steadily parallel to the rise in the concentration of the solution. The curve of relative capillarity

$\left(\frac{K_{\Delta}}{P_{\Delta}}\right)$ , which may be regarded as the curve of absorption of the solution, has the same trend as the curve of swelling with the difference that the former sinks more abruptly at high concentrations. The curve of absorption goes in the opposite direction (columnar alkali soil). With the increase in absorption of Ca, swelling decreases; therefore, the curve of absorption may be regarded as the curve of coagulation, the rise in which corresponds to the increase in the value of  $S$ , the index of "structure stability."

In the columnar alkali soil the absorption of  $Mg^{++}$  was not determined. The swelling curves ( $Wq$ ) of columnar alkali soil and of loamy chernozem differ in

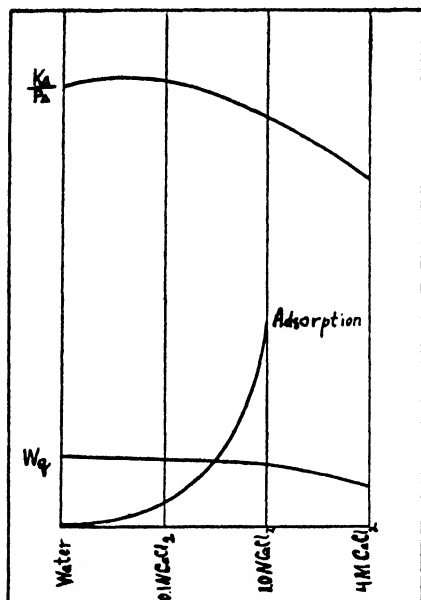


FIG. 2

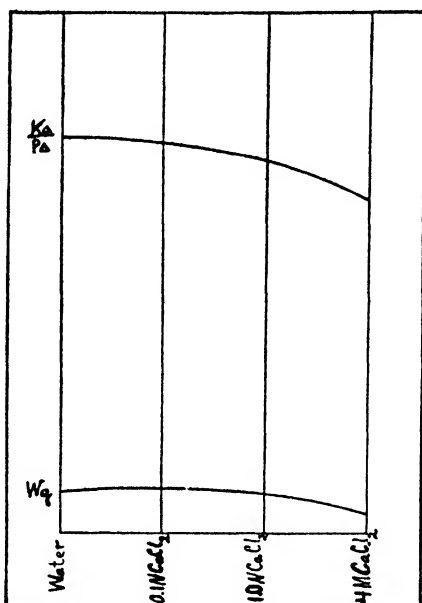
FIG. 2. SWELLING OF COLUMNAR ALKALI SOIL IN  $CaCl_2$ 

FIG. 3

FIG. 3. SWELLING OF LOAMY CHERNOZEM IN  $CaCl_2$ 

their trend. Whereas the chernozem curve descends noticeably from its maximum, which corresponds to distilled water, with the increase in concentration of the solution, that of the alkali soil has its maximum at 0.1  $N$ , then descends gradually, its  $Wq$  value at 0.5  $N$  concentration still remaining higher than that corresponding to water. For chernozem the curves of relative capillarity,  $\frac{K_{\Delta}}{P_{\Delta}}$ , have the same trend as has  $Wq$ ; for alkali soil, a slightly different one. The curve of adsorption (coagulation) of  $Mg^{++}$  has, for chernozem, an opposite trend (fig. 4 and 5).

Swelling in  $BaCl_2$  solutions is marked, as in  $CaCl_2$  solutions, by the gradual

descent of its  $Wq$  curve and the more abrupt descent of its relative capillarity curve,  $\frac{K_{\Delta}}{P_{\Delta}}$ . Thus, the  $\text{BaCl}_2$  graph resembles that of  $\text{CaCl}_2$ .

Swelling in  $\text{NaCl}$  solution produces an original curve, resembling the  $\text{MgCl}_2$  one. As with  $\text{MgCl}_2$  it reaches its maximum at 0.1  $N$  concentration, and in 0.5  $N$  solutions the value of  $Wq$  is higher than in water. The curve of absorption,  $\frac{K_{\Delta}}{P_{\Delta}}$ , has approximately the same trend as that of  $Wq$  (fig. 6 and 7).

The  $S$  index remains almost stable.

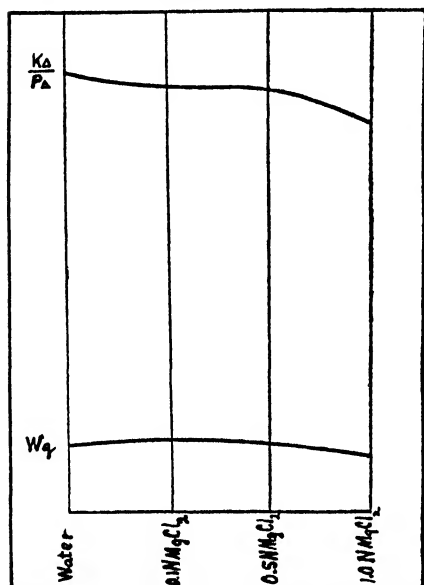


FIG. 4

FIG. 4. SWELLING OF COLUMNAR ALKALI SOIL IN  $\text{MgCl}_2$

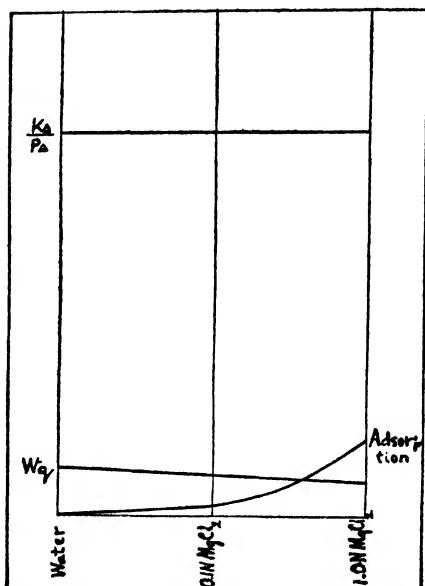


FIG. 5

FIG. 5. SWELLING OF LOAMY CHERNOZEM IN  $\text{MgCl}_2$

The swelling of columnar alkali soil in  $\text{H}_2\text{SO}_4$  decreases with the increase in acid concentration. The trend of the curves of swelling ( $Wq$ ) and of relative capillarity,  $\frac{K_{\Delta}}{P_{\Delta}}$ , is similar, as in those obtained for  $\text{CaCl}_2$  solutions.

For chernozem the trend of the swelling and water absorption curves  $\frac{K_{\Delta}}{P_{\Delta}}$  is opposite. With the increase in the absorption of acid we observe an increase in the absorption of water; this is not the case for columnar alkali soil. Apparently this increase in water absorption should be ascribed to the formation of a chemical compound with the acid or to the dissolution of the acid in soil and the formation of a solvate film (fig. 8 and 9).

The concluding experiment was conducted in  $\text{CaCl}_2$  solutions with the swelling of loamy chernozem, in which part of the replaceable  $\text{Ca}$  was replaced

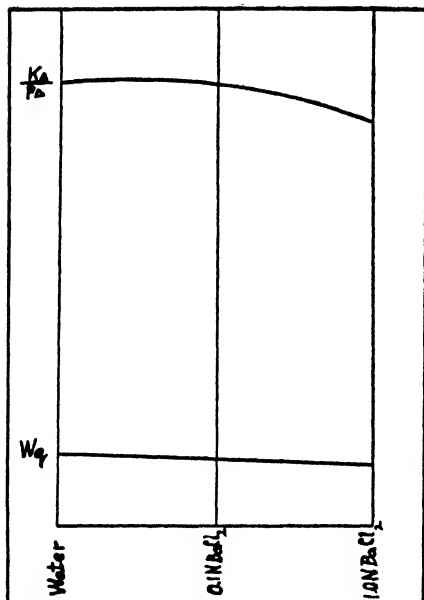


FIG. 6

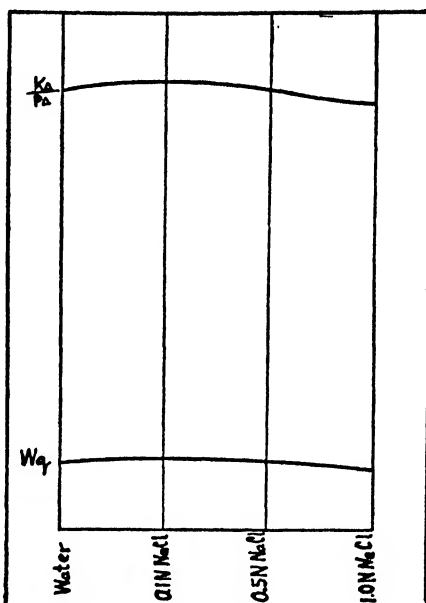
FIG. 6. SWELLING OF COLUMNAR ALKALI SOIL IN  $\text{BaCl}_2$ 

FIG. 7

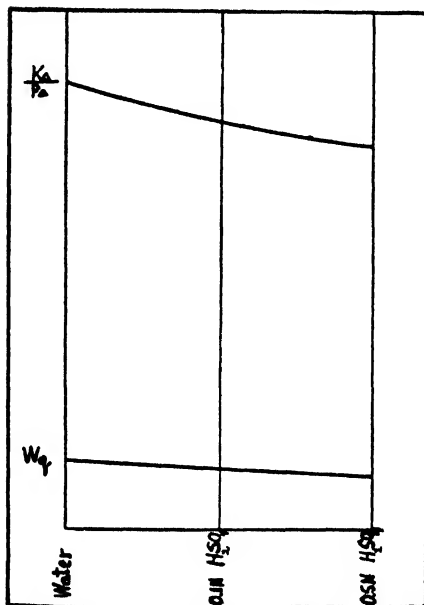
FIG. 7. SWELLING OF COLUMNAR ALKALI SOIL IN  $\text{NaCl}$ 

FIG. 8

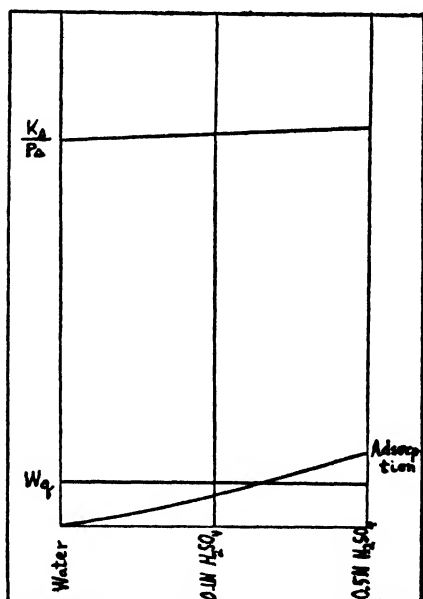
FIG. 8. SWELLING OF COLUMNAR ALKALI SOIL IN  $\text{H}_2\text{SO}_4$ 

FIG. 9

FIG. 9. SWELLING OF LOAMY CHERNOZEM IN  $\text{H}_2\text{SO}_4$

by Na to the amount of 27.94 per cent of the cation exchange capacity. Simultaneously absorption of  $\text{Ca}^{++}$  from 0.1  $N$  and 1.0  $N$  solutions of  $\text{CaCl}_2$  was studied.

A similar, though less sharp picture, than that for columnar alkali soil in  $\text{CaCl}_2$  solutions, was observed.

The trend of the swelling ( $W_q$ ) curves and of those of water absorption is here the same, the difference being that here the decrease of swelling *reveals itself in a sharper form*. As the absorption of Ca increased from 2.29 m.e. to 28.12 m.e. the swelling fell from  $W_q = 9.48$  to  $W_q = 2.42$  and 1.91 (table 16). The value of the "structure stability" index  $S$  rose correspondingly (fig. 10).

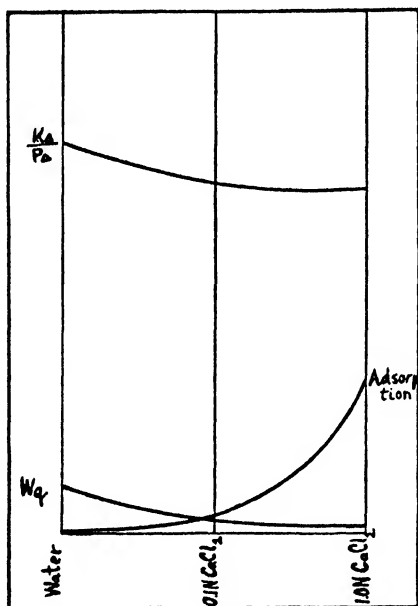


FIG. 10. SWELLING OF LOAMY CHERNOZEM SATURATED WITH  $\text{CaCl}_2$  SOLUTION WITH Ca REPLACED BY Na TO AMOUNT OF 27.94 PER CENT

The experiments with swelling in various salt solutions confirm the theory that swelling is lowered by the presence of free electrolytes and that this phenomenon may be explained with the help of the osmotic theory of swelling. The lowering of swelling is directly connected with the absorption of cations. The minimum swelling is observed at the point at which this absorption reaches its maximum. Thus in determining the swelling the curve of absorption may be considered as representing the curve of the coagulation of the colloid solution. The increase of swelling in 0.1  $N$  and 0.5  $N$  solutions of  $\text{MgCl}_2$  and  $\text{NaCl}$  as compared with swelling in distilled water may be explained by the fact that in the absorption of Mg and Na cations the electrolytic dissociation at first increases in comparison to that which took place during swelling in water and

later, after further addition of salt, it decreases. The action of acid solutions may also be explained from this point of view.

We must note further that hydration  $\left(\frac{K_{\Delta}}{P_{\Delta}}\right)$  under the influence of the Ca ion in low concentrations of  $\text{CaCl}_2$ , for instance in solutions weaker than 0.1 *N*, is greater than in water or in similar solutions of  $\text{MgCl}_2$ ,  $\text{NaCl}$ , and  $\text{BaCl}_2$ . This hydrating effect of calcium was indicated by Freundlich and Sachs for kaolin (4). Thus, in regard to hydration the position of Mg does not fully correspond to Bayer's scheme.

Absorption of Ca has a marked effect on the lowering of swelling ( $W_q$ ) only in the case of soils containing replaceable Na, e.g., columnar alkali soil, or the "artificial alkali soil" prepared from chernozem by saturating it with Na up to 27 per cent of its exchange capacity. Very characteristic of these soils is the "improvement" of their structure under the influence of free Ca ions. But this "improvement" is not the same in all soils investigated. It is less marked for columnar alkali soil than for Na-chernozem. The "structure index" rises from 0.74 (water) to 0.93 (1.0 *N* solution of  $\text{CaCl}_2$ ). This "improvement," however, takes place only under the influence of strong solutions considerably exceeding the threshold of coagulation.

In soils saturated with calcium (for instance, in loamy chernozem,<sup>2</sup> which is practically 98 per cent saturated) swelling in  $\text{CaCl}_2$  solutions proceeds almost identically with swelling in water, without any noticeable "improvement" of structure. Only in concentrated solutions (4 *M*) is a depression observed. The transformation of structural aggregates of such soils into water-tight ones by the addition of calcium salts apparently cannot be achieved. As microscopic investigation has shown, disintegration of the aggregates is very slight in concentrated solutions of  $\text{CaCl}_2$ .

A certain interest is aroused by the experiments on absorption by structureless non-swelling soils of the solutions of Ca and Mg salts and of  $\text{H}_2\text{SO}_4$  and  $\text{Na}_2\text{CO}_3$ , in which cation absorption was studied. Omitting the description of these experiments we shall note only that salts have no effect on the absorption of their solutions in this case; its value remains always equal to that of the absorption of inert liquids. This circumstance is in accordance with the idea, already developed, that in structureless soil absorption of water or of solutions is essentially a purely capillary process.

#### MICROSCOPIC RESEARCH

Rather important results have been obtained in the study of swelling by applying microscopic and cinematographic technique. A number of remarkable researches may be cited, in which the understanding of the phenomena taking place in swelling was founded on microscopic investigation of the process. Most of these works concern the swelling of vegetable fibers and of silk: such works as the investigations by Weimarn (21) of the swelling of silk; by Hess, Trogus, et al. (8), of ramie fiber swelling; by Rabinowitch (19) and

<sup>2</sup> The replaceable cations of loamy chernozem are Ca, 46.3 m.e., and Mg, 0.03 m.e.

by Hess and Rabinowitch (7) on cellulose fibers; by Beiser (2) on the swelling of fir-tree and beech fibers.

In the present investigation an attempt was made to apply the microscopic methods to the study of soil swelling. The tests were made in passing light. The objects of observation were microstructural elements  $<0.25$  mm. and  $<0.5$  mm. in size. For the sake of comparison these microaggregates were studied under the microscope both in water and in oil, magnified 50–100 times. The photographs were taken at a magnification of about 70 times.

To eliminate the possibility of compressing air (13) into the aggregates and of their bursting, they were placed into a very thin layer of water deposited on an object slide.

The phenomena of the desintegration of the aggregates, foretold on the basis of the comparative study of water and oil absorption, can be clearly seen under such conditions of the experiment. Under the microscope one may see how water penetrates into the minuscule cracks,  $<0.01$  mm., that arise during swelling and divides the microaggregates into still lesser units.

In oil the character of the phenomena observed is quite different. Here no disintegration of the aggregates or penetration of oil into the micropores is observed. The aggregates act as indivisible elements.

The photographs, taken at the same magnification, represent the microaggregates of columnar alkali soil: plate 1, figure 1, in oil, and figure 2, in water; and those of loamy chernozem: plate 1, figure 3, in oil, and figure 4, in water.

The size of the aggregates was accidentally different, but this does not disguise the different character of the absorption of oil and of water, so characteristic of structural soils as has been indicated.

In structureless soils other relations were observed. Experiments had shown that in such soils there was no difference between the absorption of oil and that of water. Therefore one could expect that under the microscope there would be no difference either. Indeed, as may be seen from plate 1, figures 5 and 6, representing the absorption of oil and that of water by loess-like loam, there is a similarity of action, consisting in the almost complete subdivision of the soil into mechanical elements. If "clods" are observed, they do not produce that characteristic picture of disintegration which is observed in structural soils. There is no splitting of aggregates (pl. 1, fig. 6), because there are no aggregates here.

As still greater evidence to prove this, we reproduce a photo showing particles of loess-like loam in a saturated solution of  $\text{CaCl}_2$  (pl. 1, fig. 7). As was demonstrated earlier in the chapter on swelling in solutions of electrolytes, saturated solutions of  $\text{CaCl}_2$  produce a considerable lowering of swelling. This was confirmed for structural soils by microscopic tests. The aggregates of such soils (see hereinafter) do not split at all or show but slight disintegration when placed in a saturated solution of  $\text{CaCl}_2$ . One would expect, therefore, that in a saturated solution of  $\text{CaCl}_2$  the aggregates of loess-like loam, if they

exist, must remain intact. Figure 7, plate 1, however, shows a picture identical to that of figure 6, as if the loess-like loam in it was not in  $\text{CaCl}_2$  but in water.

Thus microscopic investigation confirms the conclusion, arrived at earlier, that absorption of polar and non-polar liquids permits sharp distinction between structural and structureless soil and that water absorption by structural soil is closely connected with an unsettling of this structure and an increase in the degree of dispersion of this soil.

Simultaneously with the study of water absorption, microscopic investigation of the influence of solutions of electrolytes on the aggregates of structural soils was conducted.

As can be seen from the foregoing discussion, the action of salt solutions lowers the swelling. This lowering proved, however, to be rather small, as compared with swelling in water, even for 1.0 *N* solutions. Only in the case of the saturated solution of  $\text{CaCl}_2$  were a considerable lowering of swelling and an almost complete "restoration" of structure noted. Microscopic investigation confirms these results. Figures 1 and 2, plate 2, represent the swelling of columnar alkali soil aggregates in 1.0 *N* and in saturated solutions of  $\text{CaCl}_2$ .

Figures 3, 4, and 5, plate 2, represent the swelling of loamy chernozem in 0.1 *N*, 1.0 *N*, and saturated solutions of  $\text{CaCl}_2$ . It may be observed in them that the disintegration of alkali soil aggregates in 1.0 *N* solutions of  $\text{CaCl}_2$  is somewhat less than in water (pl. 1, fig. 2).

The same thing may be observed for loamy chernozem also (fig. 3 and 4, plate 2). In saturated solutions of  $\text{CaCl}_2$  the aggregates of both the columnar alkali soil and the chernozem (fig. 2 and 5, plate 2) show considerably less splitting than in 1.0 *N* solution of  $\text{CaCl}_2$ . This is in good agreement with the values we found for the "swelling solution" (*Wq*).

Figures 6 and 7, plate 2, represent respectively the swelling of columnar alkali soil and loamy chernozem in a normal solution of  $\text{MgCl}_2$ . Here, in contradistinction to  $\text{CaCl}_2$ , hydration (disintegration) is, as has already been noted, less in the same degree in both alkali (fig. 6, pl. 2) and chernozem (fig. 7, pl. 2) soils. The latter circumstance agrees with the results obtained for  $\text{MgCl}_2$  in the swelling experiments.

Figures 1 and 2, plate 3, represent the swelling of columnar alkali soil and loamy chernozem in 0.5 *N* solution of  $\text{H}_2\text{SO}_4$ . The disintegration of the chernozem aggregates (fig. 2, pl. 3) is almost the same as in water; that of the alkali soil aggregates (fig. 1, pl. 3) is somewhat less. This also is in agreement with the results of the experiments.

#### X-RAY INVESTIGATION

It was noted at the beginning of this article that the application of X-ray analysis to the study of swelling has acquired the importance of a method, revealing the essence of structural changes in almost every case in which the swelling substance is composed of the crystalline phase. The most detailed research work of this kind was done by Katz and Mark (11) on cellulose, by



Hofmann and Endell (9) on the swelling of the argillaceous mineral montmorillonite, and by Hofmann and Frenzel (10) on the swelling of graphitic acid.

The purpose of the present investigation was to elucidate the character of the structural changes in the soil during swelling. The supposition that soil suspensions consist of crystalline substance was based on the works of Hendricks and Fry (5) and of Kelley, Dore, and Brown (12).

The first roentgenoscopic determinations<sup>3</sup> concerned a sample of columnar alkali soil the components of which were kept undivided. The roentgenograms were obtained by the Debay method in chromium rays with a wavelength of  $K_{\alpha} = 2.287 \text{ \AA}$  and  $K_{\beta} = 2.08 \text{ \AA}$ , in a chamber 57.4 mm. in diameter.

To protect the swollen soil sample from evaporation during the roentgenographic operation, which lasted 9 hours, it was put into a thick celluloid tube. The soil did not dry out in such a tube during 48 hours. The lines obtained in the roentgenograms (fig. 3 and 4, pl. 3), with the possible exception of one, coincide with the lines of crystalline quartz.

No difference can be observed between dry and swollen soil in these roentgenograms. This led us to suppose that, besides quartz, there may be other minerals in soil capable of taking part in swelling but that they are found in soil in such small quantities, as compared with quartz, that they cannot produce the corresponding interferences in the roentgenogram.

To verify this, a suspension was isolated from columnar alkali soil, consisting of particles smaller, and for the most part much smaller, than  $1 \mu$ . In investigating these suspensions—both dry and swollen—(figs. 5, 6, 7, and 8, pl. 3) either no lines were found or there appeared a system of weak lines of crystalline quartz. Besides these, two weak lines are observed in both the dry and the swollen suspensions. Besides the suspension of columnar alkali soil, another suspension isolated from loess-like loam was also tested roentgenographically. This suspension, like that of columnar alkali soil, produced identical roentgenograms in dry and swollen condition. A similar roentgenogram was produced by the suspension of columnar alkali soil swollen in 1.0 *N* solution of  $\text{CaCl}_2$  (fig. 9).

The results show that suspensions of columnar alkali soil and of loess-like loam contain an insignificant quantity of crystalline quartz and some other mineral, the nature of which we were not able to determine by the two weak lines it produced. It should be noted that these lines do not coincide with those of montmorillonite, bentonite, and galloisite, i.e., of typical mineral soil colloids.

Thus in the samples tested crystalline substance does not take any part in the swelling process. The main mass of the suspension of columnar alkali

<sup>3</sup> The roentgenoscopic study of swelling was conducted in the Institute for Fireproof Materials, Kharkov, under the supervision of the chief of the X-ray laboratory of this institute, B. J. Pines, to whom I consider it my duty to express my thankfulness for valuable aid and suggestions.

soil consists of amorphous substance, which is responsible for the changes connected with soil swelling.

#### SUMMARY

The influence of solutions of electrolytes on swelling was studied. It was found that solutions of salt and of  $H_2SO_4$  lower swelling, with the exception of 0.1 *N* and 0.5 *N* solutions of NaCl and  $MgCl_2$ .

Parallel with the study of the swelling of soil in solutions of electrolytes, a study was made of the absorption of cations. It was found that the curve of absorption has an opposite trend to the curve of swelling. The curve of absorption may be regarded as the curve of the coagulation of the sol of the soil colloid.

Microscopic investigation confirmed the method by which a sharp difference between structural and structureless soil was established on the basis of absorption of water and of inert liquids by soil.

Microscopic investigation of swelling in solutions of electrolytes gives results coinciding with the values found for "swelling solution." This confirms the correctness of the choice of "swelling water" ("swelling solution") as an indicator determining the process of swelling in its proper quality.

Roentgenographic investigation of suspensions isolated from structural and structureless soils showed that they contain but slight amounts of crystalline substance, the prevalent mineral of which is quartz. It is demonstrated that crystalline substance takes no part in the process of swelling. The main mass of the suspension of columnar alkali soil consists of amorphous substance with which the process of change is closely connected.

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#### PLATE 1

- FIG. 1. Columnar alkali soil in oil.
- FIG. 2. Columnar alkali soil in water.
- FIG. 3. Loamy chernozem in oil.
- FIG. 4. Loamy chernozem in water.
- FIG. 5. Loess-like loam in oil.
- FIG. 6. Loess-like loam in water.
- FIG. 7. Loess-like loam in saturated  $\text{CaCl}_2$ .



FIG. 1

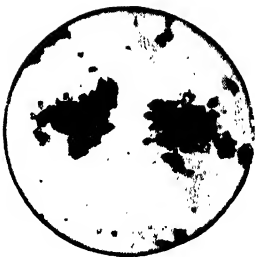


FIG. 2

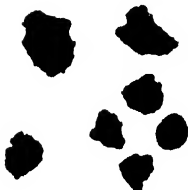


FIG. 3



FIG. 4



FIG. 5



FIG. 6



FIG. 7

## PLATE 2

- FIG. 1. Columnar alkali soil in 1.0 *N* CaCl<sub>2</sub>  
FIG. 2. Columnar alkali soil in saturated CaCl<sub>2</sub>  
FIG. 3. Loamy chernozem in 0.1 *N* CaCl<sub>2</sub>  
FIG. 4. Loamy chernozem in 1.0 *N* CaCl<sub>2</sub>  
FIG. 5. Loamy chernozem in saturated CaCl<sub>2</sub>  
FIG. 6. Columnar alkali soil in 1.0 *N* MgCl<sub>2</sub>  
FIG. 7. Loamy chernozem in 1.0 *N* MgCl<sub>2</sub>.



FIG. 1



FIG. 2

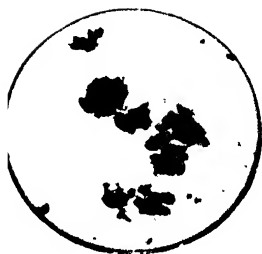


FIG. 3

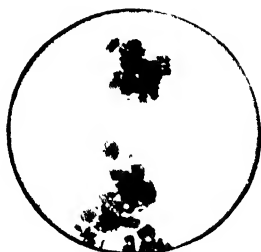


FIG. 4

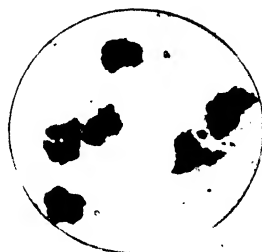


FIG. 5



FIG. 6

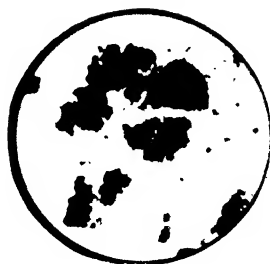


FIG. 7

## PLATE 3

FIG. 1. Columnar alkali soil in 0.5 *N* H<sub>2</sub>SO<sub>4</sub>.

FIG. 2. Loamy chernozem in 0.5 *N* H<sub>2</sub>SO<sub>4</sub>.

FIGS. 3-9. Roentgenograms of dry and swollen columnar alkali soil



FIG. 1



FIG. 2

FIG. 3



FIG. 4



FIG. 5



FIG. 6



FIG. 7



FIG. 8



FIG. 9







## STUDIES ON PODZOLS AND BROWN FOREST SOILS: II

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In the first part of this paper it was demonstrated that podzols and brown forest soils differ not only in their morphological but also in their chemical nature (3). Tamm's acid-oxalate method proved to be very useful for the study of those differences, but there are several other ways of demonstrating them. Some of the ways of showing evidences of podzolization have been discussed by Joffe (1), and Mattson has recently pointed out that "the most rational method is to be found in a determination of the amphoteric points of the respective complexes" of the different horizons in the soil profile (13, p. 472). The conception of the soil colloids as ampholytoids (10) led to the theory of isoelectric weathering (11), according to which the composition and the properties of the colloids depend on the type of weathering prevailing in the soil.

In the case of the podzols the weathering can be considered as a typical acid hydrolysis. The colloidal residue in the upper, acidic horizons (A) of a podzol profile is strongly acidoid, whereas the basoids (or rather ampholytoids), being ionized, move farther down with the percolating water. In the B horizons where the sol encounters a higher prevailing pH it is precipitated, and the gel formed has a less acidoid (more basoid) character than the colloids left in A. The opposite type of weathering, a basic hydrolysis, is represented by the laterites. Here the pH in the upper part of the profile is high enough to prevent the ionization and dispersion of the ampholytoids, whereas the acidoids move downward, leaving a residue high in sesquioxides. In the case of the brown forest soils it has been demonstrated that the colloids, especially the sesquioxides, as determined by the acid-oxalate method, are evenly distributed in the A and B horizons of the profile or have their maximum in A (2, 3). This would indicate, from the standpoint of isoelectric weathering, that these soils are formed under circumstances causing a weathering of less acid type than the podzols where the sesquioxides are removed from the colloid complex of the A horizons.

In the following pages are given the results of a study of the amphoteric

<sup>1</sup> The laboratory work involved in this study was done by the author in the laboratory of Dr. Sante Mattson during a stay as Cook-Voorhees Research Fellow at the New Jersey Agricultural Experiment Station October, 1931–September, 1932 [see acknowledgment in the first part of this paper (3)].

reactions of the soil from the various horizons of podzol and brown forest soil profiles. The profiles are the same as those used in the first part of this paper (3, p. 146-148), that is, profile 1 is the typical brown forest soil from Hissö, province Småland, Sweden; profile 2 is a podzol from the same province; profile 3 is an aclimatic brown forest soil from Siljansfors, province Dalarna, Sweden; profiles 4, 5, and 6 are typical iron podzols from different plant communities at Siljansfors; and profile 7, from the same place, is a podzol related to the iron humus podzol of Tamm (14, p. 366).

#### IONIC EXCHANGE

The ion exchange capacity, that of both the cation and the anion, is a most significant expression of the nature of colloidal complexes. It has been demonstrated that the cation exchange capacities at pH 7 increase with the proportion of acidoid to ampholytoid in the complex and also that in the same type of complexes the exchange capacity is higher the weaker the basic properties of the ampholytoid present (9). This is exactly what the theory of the amphoteric nature of colloids requires and must be true at any given pH down to the point of exchange neutrality. For the anion exchange capacity the rule must be the opposite: it decreases with an increasing acidoid ampholytoid ratio.

Let us now see how the ion exchange capacities should vary in the different layers of soils.

Take first the cation exchange capacity. If the theory of isoelectric weathering be true the base exchange per gram colloid should be higher in a podzol than in a soil formed by a less acid type of weathering. And in a podzol there should be a higher base exchange in the A horizons than in the B because the ampholytoids, such as the sesquioxides, are leached out from A and re-deposited in B, thus making the colloids there less acidoid. But now we must remember that the ion exchange capacity, determined on the whole soil, is not only a qualitative but also a quantitative property. It is well known—and was proved in the first part of this paper—that the colloids are generally far more abundant in B than in A. Despite the fact that the mineral *colloid complex* in the A horizons of a podzol profile has a much higher base exchange capacity than the one in B it can therefore very well happen that *the whole soil* has a lower base exchange in A than in B. If this be the case, and as will be shown later it is generally true for the A<sub>2</sub> horizons, it is a proof of the poverty of colloids in these horizons. From this discussion it is clear that the base exchange capacity, because of its qualitative and quantitative nature, cannot be used as a means of characterizing the type of soil formation unless some other determinations are made to show the acidoid/ampholytoid ratio of the complexes.

What will be the result of determinations of the anion exchange capacities? Here we must find that the more the ampholytoid prevails in the complex and the more basic its character, the higher is the anion exchange capacity.

TABLE 6

*Isoelectric point, exchange neutrality, ultimate pH, base exchange, combining capacity at pH 7, base saturation, and actual pH of samples from the different horizons of podzols and brown forest soils*

PROFILE NUMBER	SOIL TYPE	HORIZON	ISOELECTRIC POINT	ULTIMATE pH	pH OF EXCHANGE NEUTRALITY	BASE EXCHANGE, M.E. PER 100 GM.	COMBINING CAPACITY AT pH 7, M.E. PER 100 GM.	BASE SATURATION AT THE pH OF THE NATURAL SOIL, PER CENT	pH OF THE NATURAL SOIL	ELECTRODIALYZABLE BASES, M.E. PER 100 GM.
1	Brown forest soil, typical	A	...	4.1	4.0	25.93	11.5	63	6.1	4.47
		B <sub>1</sub>	...	4.8	4.4	12.85	10.5	26	5.7	0.74
		B <sub>2</sub>	3.7	5.4	5.1	9.18	5.7	31	5.9	0.42
		C <sub>1</sub>	3.9	5.4	5.5	4.33	1.5	50	6.1	0.34
		C <sub>2</sub>	3.9	5.4	5.3	7.19	2.2	45	5.9	0.24
2	Podzol	A <sub>2</sub>	...	4.0	4.0	19.20	7.5	-7	4.0	0.15
		B <sub>1</sub>	4.0	5.4	5.3	18.17	13.0	-19	4.7	0.09
		B <sub>2</sub>	4.0	5.4	5.7	10.11	4.8	-94	4.9	0.51
		C <sub>1</sub>	5.1	5.4	6.0	15.20	2.0	-75	5.3	0.19
		C <sub>2</sub>	5.2	5.5	6.3	2.18	0.2	-50	6.2	0.07
3	Brown forest soil, acclimatic	A	...	4.3	4.1	107.00	circ. 60.0	circ. 50	4.8	28.40
		B <sub>1</sub>	...	4.6	4.8	10.34	7.5	57	6.1	0.49
		B <sub>2</sub>	...	4.5	5.0	3.93	2.5	70	6.2	0.55
		B <sub>3</sub>	...	4.5	4.8	6.91	3.9	77	6.2	0.22
4	Iron podzol	A <sub>2</sub>	...	3.9	3.9	4.29	3.0	-17	4.1	0.08
		B <sub>1</sub>	4.1	5.7	5.6	9.36	3.5	36	6.1	0.10
		B <sub>2</sub>	4.1	5.8	5.7	4.51	2.3	22	6.1	0.71
5	Iron podzol	A <sub>2</sub>	...	4.2	4.1	3.23	5.0	5	4.5	0.94
		B <sub>1</sub>	4.2	5.1	5.0	11.14	20.0	13	5.8	0.95
		B <sub>2</sub>	4.3	5.6	5.4	2.97	2.3	33	6.1	0.91
		C <sub>1</sub>	4.8	4.6	5.3	1.32	1.0	50	6.2	0.86
		C <sub>2</sub>	4.7	4.5	5.3	1.39	1.0	50	6.2	0.59
		C <sub>3</sub>	...	4.5	5.3	1.63	1.0	50	6.2	0.67
6	Iron podzol	A <sub>0</sub>	...	3.5	3.0	114.88	20.0	....	3.8	10.55
		A <sub>2</sub>	...	4.1	4.0	6.54	4.5	0	4.1	0.52
		B	5.3	5.7	6.0	7.52	3.2	8	6.1	0.26
		C	5.0	5.9	6.5	1.10	0.2	-50	5.9	0.17
7	Iron humus podzol	A <sub>2</sub>	...	4.2	4.1	5.87	3.5	23	4.7	0.42
		B <sub>1</sub>	3.7	5.4	4.6	38.63	25.0	20	5.7	0.30
		B <sub>2</sub>	3.8	4.9	4.8	28.92	29.0	23	5.7	0.28
		C	3.4	4.7	5.3	2.37	2.7	28	5.9	0.35

In a podzol profile we should therefore expect to find a higher exchange capacity in the B horizons, with their accumulation of sesquioxides and other ampholytoids, than in the A horizons. In the case of a very marked basic type of weathering we could perhaps have somewhat the same difficulty in interpreting the results as in the case of the base exchange in the podzols. But generally the anion exchange capacities of the different horizons would well characterize the type of soil formation. In any case a combination of determinations both of the anion and the cation exchange capacities would give good information of the soil type and the nature of the colloids and, to a certain extent, even give a relative measure of the amounts of colloids present.

Determinations of the base exchange were made in the following way. Five grams of air-dry soil, passed through a 2-mm. sieve, was put in a beaker, some neutral *N* barium acetate solution was added, and the contents were gently stirred. After some time the liquid and the soil were transferred to a filter and then the soil was leached with small portions of the barium acetate solution until the filtrate showed neutral<sup>2</sup> reaction (in all cases brom thymol blue was used as an indicator), and then a few times more. A total volume of 250 cc. was always used. The filter was then filled with neutral *N* barium chloride solution (10 cc.), and, when this had passed through, the residue was washed with cold distilled water until the filtrates showed no chloride reaction. All "exchangeable bases" are then supposed to be displaced by barium and all "free" barium salts removed by washing. The residue on the filter was then treated with small portions of *N* ammonium chloride solution at a temperature of 70°C. In all, 250 cc. of this solution was used for each sample. The barium was determined as BaSO<sub>4</sub> in the common way. The results, are shown in table 6 where the Ba displaced by NH<sub>4</sub>, the base exchange capacity, is given in milliequivalents per 100 gm. of oven-dry soil (105°C.). They will be discussed later in connection with the points of exchange neutrality and will here merely be summarized as follows. The base exchange of the brown forest soils decreases from the A to the B and C horizons. In the podzols two clear maxima of base exchange occur, one in A<sub>0</sub>, because of its high humus content, and one, though much smaller, in the upper part of B; this depends on the higher amount of colloids in B as compared to A<sub>2</sub> and C, where the actual colloids present must have the highest exchange capacity in the A horizon. The results obtained from the different iron podzols seem to confirm the previously expressed idea that the acid-oxalate method gives a measure of the reactive colloids. At least the differences in exchange capacity can be explained from the composition of the colloids thus determined.

<sup>2</sup> Whether the determinations be made at pH 7 or at any other pH above the point of exchange neutrality is naturally entirely arbitrary, provided all determinations be made at the same pH; there will always be a difference between the soils depending on the composition and the amounts of colloids present. But a linear relationship between base exchanges determined at different pH values can not be expected because of the possible differences in the linkage within the micellae of the colloid complex at different reactions.

## CATAPHORESIS

The isoelectric point has been found to vary with the composition of the soil colloidal complex in such a way that this point lies at a higher pH the higher the basoid (sesquioxide) constituents of the complex, whereas a complex having a high acidoid (silica, humus) content is isoelectric at a low pH. Since the isoelectric complex represents its most stable condition we should, according to the theory of isoelectric weathering, under conditions of a heavy leaching and a high maturity, expect to find the composition and therefore also the isoelectric point of the soil colloid corresponding to the prevailing pH of the soil. In the case of many of the natural soils the isoelectric weathering is more of a tendency to an ultimate condition than a fully established fact. We must remember that colloids flocculate not only at the isoelectric point but also in a more or less wide range of pH in the neighborhood of this point (7). In any case the isoelectric point indicates the nature of the colloids in the soil and should give information about the type of weathering prevailing.

The isoelectric point was determined by cataphoresis measurements, using the apparatus described by Mattson (6). The air-dry soil samples (5 gm. of each) were shaken in rubber stoppered test tubes with 0.01 *N* NaCl solution containing different amounts of HCl. The reason for using NaCl solution instead of pure water for the suspension is that the salt so hastens the attainment of equilibrium that the measurements can be made the day after mixing (13, p. 472). After repeated shaking the test tubes were left undisturbed for a day. Then small samples of the clear solutions were taken for colorimetric pH determination and the rest were immediately gently shaken and used for the cataphoresis measurements.

It is doubtful, however, whether the soils had assumed their final charge. Since they had been kept dry for a considerable time it can be assumed that the gels were "aged" to an extent that they were acted upon too slowly by so dilute acid. Cataphoresis measurements should be made on moist soils, not on dried samples. Furthermore, cataphoresis is not always reliable for a determination of the isoelectric point because all inert substances charge themselves negatively in water. An ampholytoid which does not possess pronounced amphoteric properties may not develop a strong enough positive charge to become isoelectric at the chemically true isoelectric point.

For these reasons, and in order to save space, the entire results of the cataphoresis measurements will not be reported here. Only the isoelectric points thus determined (partly interpolated values) are reported (table 6). In the case of some of the A horizons no determination was possible, because of the formation of gas bubbles at the electrodes. In the case of profile 3 determinations were hardly possible in any horizon.

## ELECTRODIALYSIS

The electrodialyses were made in the Mattson three-compartment cell, as thin a layer of soil as possible being used to shorten the time of electrodialysis

(4). The cell was placed in a 110 volt D.C. circuit in series with three 100-watt lamps placed in parallel. The water in the electrode chambers was changed at intervals of some hours, and when the experiment had proceeded so far that 2 or 3 hours' continued electrodialysis gave no reaction for bases with phenolphthalein the electrodialysis was considered complete. Generally it was so after 24 hours or a little more. In the case of the A horizons of the brown forest soils (profiles 1 and 3) a considerably longer time was required (45 and 48 hours). On the other hand the A<sub>0</sub> layer from the podzol profile 6 was very easily electrodialyzed though it contained fairly large amounts of bases. In fact, it was completely electrodialyzed after 4 hours, but in spite of that, the electrodialysis was continued for 24 hours as in the case of most of the other soils.

The amounts of bases made free by electrodialysis were determined by titration. An attempt was made to determine the diffusible anions also, but this was very often found to be extremely difficult because of humus coloration of the solutions. No exact values of the amounts of "adsorbed" anions can therefore be given. The amounts of electrodialyzable bases found are reported in table 6.

There is a very marked difference in the amounts and the occurrence of electrodialyzable bases in the brown forest soils and in the podzols. In the typical brown forest soil we find a fairly high amount of such bases in the mull, a much lower amount in the upper B horizon, and a continually decreasing amount in the deeper part of the profile. The acclimatic brown forest soil shows these same characteristics except that there is a much more marked maximum in the mull. To a certain extent the same distribution occurred in the podzols: the highest amount of bases is found in the humus layer, a much lower one in A<sub>2</sub>, and generally a still lower one in B, but often an increase is again recorded in the deeper parts of B.

A comparison between the amounts of electrodialyzable bases found in brown forest soil and in podzol profiles from the same region and on the same parent material is of great interest. It was found, by comparing profiles 1 and 2 with 3 and 4, that the brown forest soils contain much higher amounts of bases. It is certain that this is one of the most important factors for maintaining the soil and vegetation types. On the other hand, the higher percentage of bases in the litter from a natural wood on brown forest soils tends to keep the soil in good condition, whereas a change in the vegetation, yielding a litter poorer in bases, can cause a degradation of the brown forest soil (2).

The high amounts of bases found in profile 5 as compared to the other iron podzols from the same place, profiles 4 and 6, can also be correlated with the good growth of the trees and the comparatively rich ground vegetation on the sample plot. In fact the vegetation type—the *Dryopteris*-type—where this profile was taken is the richest of all vegetation types on typical iron podzol in the region and, from the standpoint of the forester, one of the most productive.

## ULTIMATE pH

Ultimate pH is the pH of the completely electrodialed material according to Mattson's definition (10). This pH depends upon the strength of the acidic and basic residues of the soil complex and is therefore closely related to the isoelectric point.

The ultimate pH was determined in the following very simple way. The completely electrodialed soil was shaken in a rubber-stoppered test tube with freshly boiled distilled water. The ratio of dry electrodialed soil to water was kept as narrow as possible (1:2). In the case of very humus-rich soils a wider ratio is necessary in order to get any liquid for the determination. Thus the ratio was 1:5 for the A horizon of profile 3 and 1:8 for A<sub>0</sub> of profile 6. After repeated shaking for a day the test tubes were left undisturbed for 2 or 3 days. After that time the supernatant liquids were clear enough to allow colorimetric pH determinations. These were made in the Hellige-Klett comparator with standard color discs and indicators. The indicators used were yellow, brom cresol green, brom cresol purple, and brom thymol blue. If a sample showed a pH within the range of two indicators it was determined with both, and the results were always the same. At the same time the actual pH values of the original, not electrodialed, soils were determined in exactly the same way. These were reported in the first part of this work but are quoted here again as a comparison with the ultimate pH. The results are seen in table 6.

The figures show that the ultimate pH is generally lower than the actual pH of the soil. This means that the soils are at least partly "saturated" with bases, which naturally must make the pH higher. Thus the bases present in the soil exert a certain resistance against the isoelectric weathering, and the freer the soils are from bases the more typical and responsive to the climatic conditions they are. The exceptions to the rule—some samples from profile 2—seem difficult to explain unless it be that this soil contains much soluble acids which go through the membrane during the electrodialed.

The ultimate pH is lowest in the A horizon and generally increasing through the B and C horizons. This is true in most cases, but in two of the podzols (profiles 5 and 7) there is a maximum in the B horizon. There are only small differences in ultimate pH between the two soil types. The main difference is in the A horizon. The mull of the brown forest soils has an ultimate pH a little above 4. In the podzols, at least the upper of the corresponding layers, A<sub>0</sub> and A<sub>2</sub>, is more acid, even as low as 3.5 in the case determined. The A<sub>2</sub> horizon has an ultimate pH about equal to that of the mull of the brown forest soils. A comparison between soils of the different types from localities near one another—profiles 1 and 2 and profiles 3 and 4—shows a slightly higher ultimate pH for the mull than for the podzol A<sub>2</sub> horizon, but the difference is so small that its significance seems uncertain.

Thus the ultimate pH gives a confirmation of the close relationship between



podzols and brown forest soils and at the same time shows a clear difference between them in the properties of the A horizon.

#### EXCHANGE NEUTRALITY AND COMBINING CAPACITY

When treated with a neutral salt solution, a completely unsaturated soil colloid, i.e., a colloid in which all diffusible anions and cations have been substituted by OH and H ions, for instance through electrodialysis, generally gives an exchange acidity. If the pH of the solution is adjusted by adding some free acid, a point can be reached where the colloid does not affect the reaction of the solution. This pH, according to Mattson's definition, is called "the point of exchange neutrality." The reason for the unchanged reaction is that at this point the displacement of OH ions by the anions of the salt just balances the displacement of H ions in the complex by the cations of the salt (10, p. 352). If more acid is added, the solution plus colloid will be less acid than the pure solution: the colloid gives an exchange alkalinity. Above the point of exchange neutrality the effect of the colloid is an exchange acidity, i.e., if alkali is added to the neutral salt solution the solution will have a lower pH in the presence of the colloid. In this way curves can be construed which give much valuable information about the properties of the colloid (13).

The form of the curves will depend on two things: the amount of the colloid present and its composition. The combining capacity for bases depends on the number of acid equivalents in the complex which are strong enough to be neutralized at the pH in question. This amount is naturally increased with increasing amounts of soil present, or, which is the same thing, with an increasing percentage of colloid in the material. Only one point will be the same: the point of exchange neutrality. This is, as seen from its definition, a qualitative property of the colloid and it is therefore constant within reasonable limits of concentration. This fact was proved experimentally by Mattson and Hester (13, p. 467).

From the curves we can immediately read the amount of base adsorbed at any pH above the point of exchange neutrality. At pH 7 we get, for instance, an exchange capacity comparable with the one determined in the common way. From the actual pH of the soil and the corresponding pH on the curve we can also obtain an estimate of the degree of saturation of the soil as well as its lime requirement. As pointed out by Mattson and Hester, the curves can be used for determining the amount of colloid present in the soil (13, p. 463).

Figures 6 to 12 show the results of determinations of exchange neutrality and combining capacity of samples from the different horizons of the seven profiles here previously described and discussed. The experiments were carried out exactly in the same way as described by Mattson and Hester, i.e., samples of the electrodialyzed soils were shaken in test tubes with  $N$   $\text{Na}_2\text{SO}_4$  solution containing different amounts of sodium hydroxide and sulfuric acid respectively. In each case 2 gm. of the electrodialyzed soil, calculated on the

oven-dry basis, was used, the volume of the solution being 10 cc. The small amount used for each determination is naturally a great source of error in the

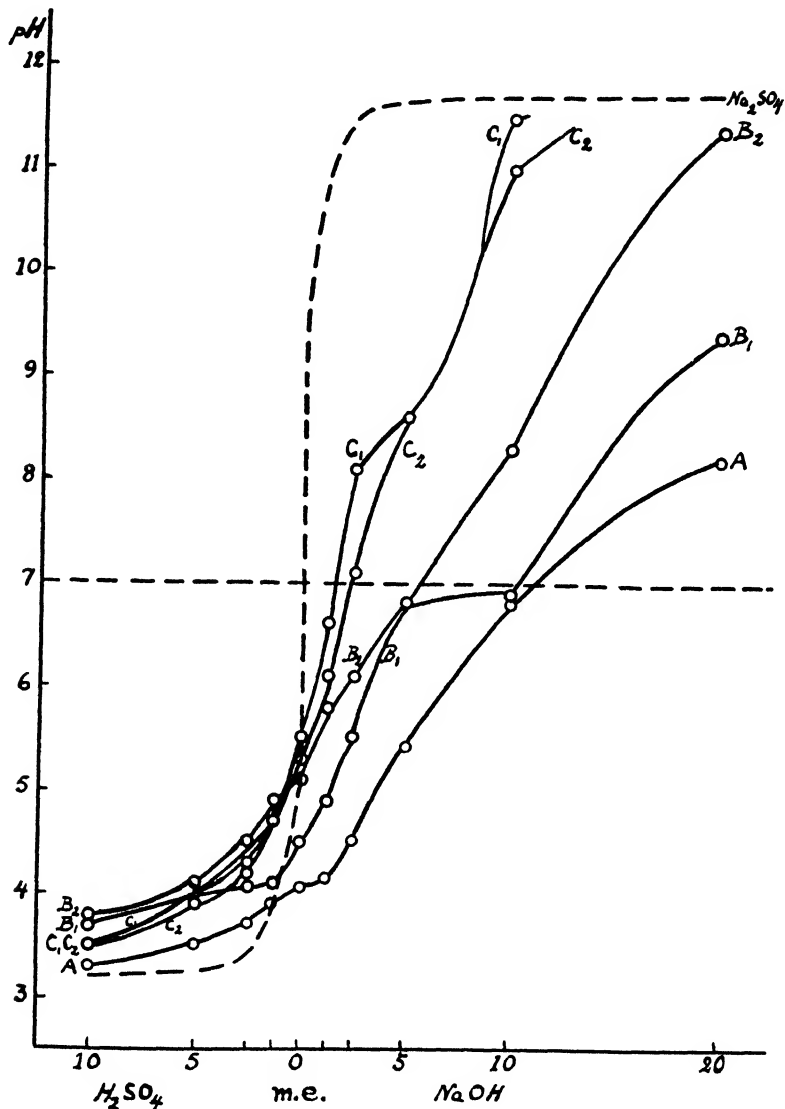


FIG. 6. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF PROFILE 1 (TYPICAL BROWN FOREST SOIL)

case of these coarse soils. Larger amounts could not be taken, however, because of the comparatively small samples of the material at hand. After repeated shaking for some hours the test tubes were left undisturbed over-

night, and then colorimetric pH determinations of the clear solutions were made in the same way as described in the chapter on the ultimate pH. The indicators used were yellow, brom cresol green, brom cresol purple, brom

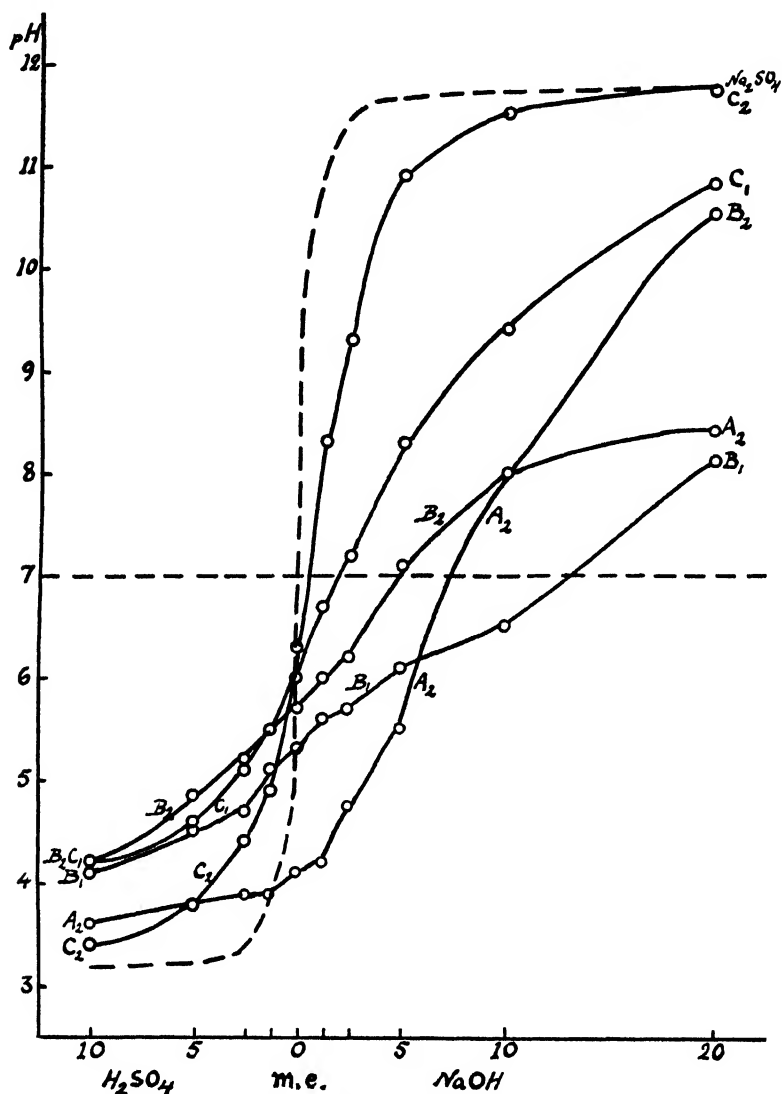


FIG. 7. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF PROFILE 2 (PODZOL)

thymol blue, phenol red, thymol blue, thymol phthalein, alizarine yellow. Determinations were always made with two different indicators if the pH was in the range of both. The points of exchange neutrality, as read from the curves, are also to be found in table 6.

It is immediately seen that the tendency to variation in the pH of exchange neutrality is exactly the same as in the ultimate pH. There is always a low

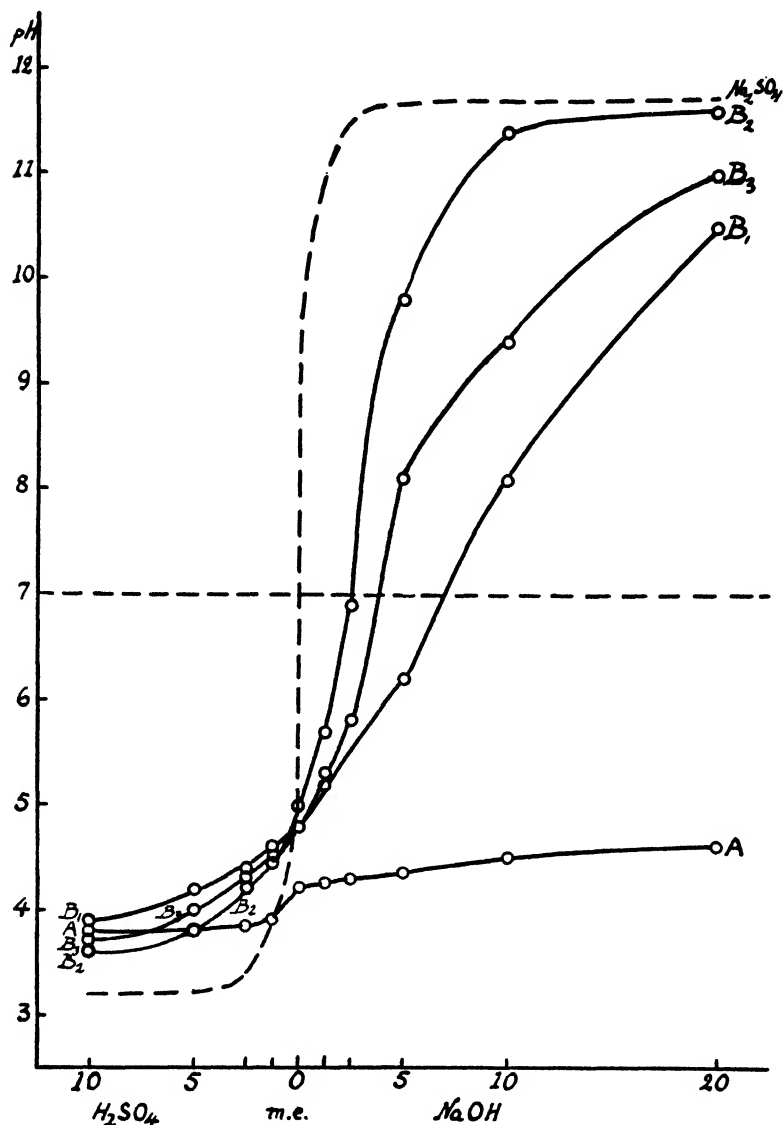


FIG. 8. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF PROFILE 3 (ACCLIMATIC BROWN FOREST SOIL)

pH of exchange neutrality in the A horizons. Generally it increases through the B horizons and is highest in C, in accordance with the theory. In the soils here investigated, the pH of exchange neutrality is often lower than the

ultimate pH. This is contrary to the results of Mattson and Hester (13, p. 462), but those results were obtained with soils very much richer in colloids.

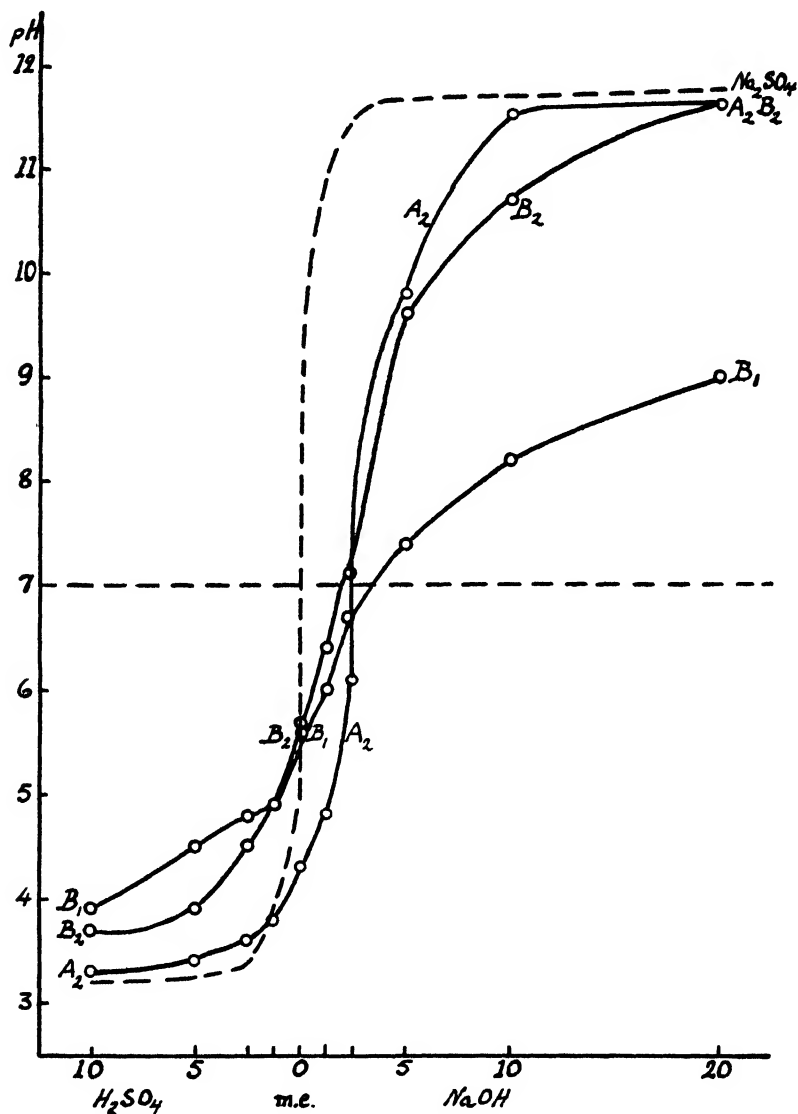


FIG. 9. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF PROFILE 4 (IRON PODZOL)

It is therefore, easily explained: the dilution of the colloid when the ultimate pH is being determined is fairly high in the cases now reported and therefore the pH found must be somewhat higher than the real ultimate pH. There

are, however, several cases in the lower horizons where the pH of exchange neutrality is higher than the ultimate pH. This would indicate a high adsorp-

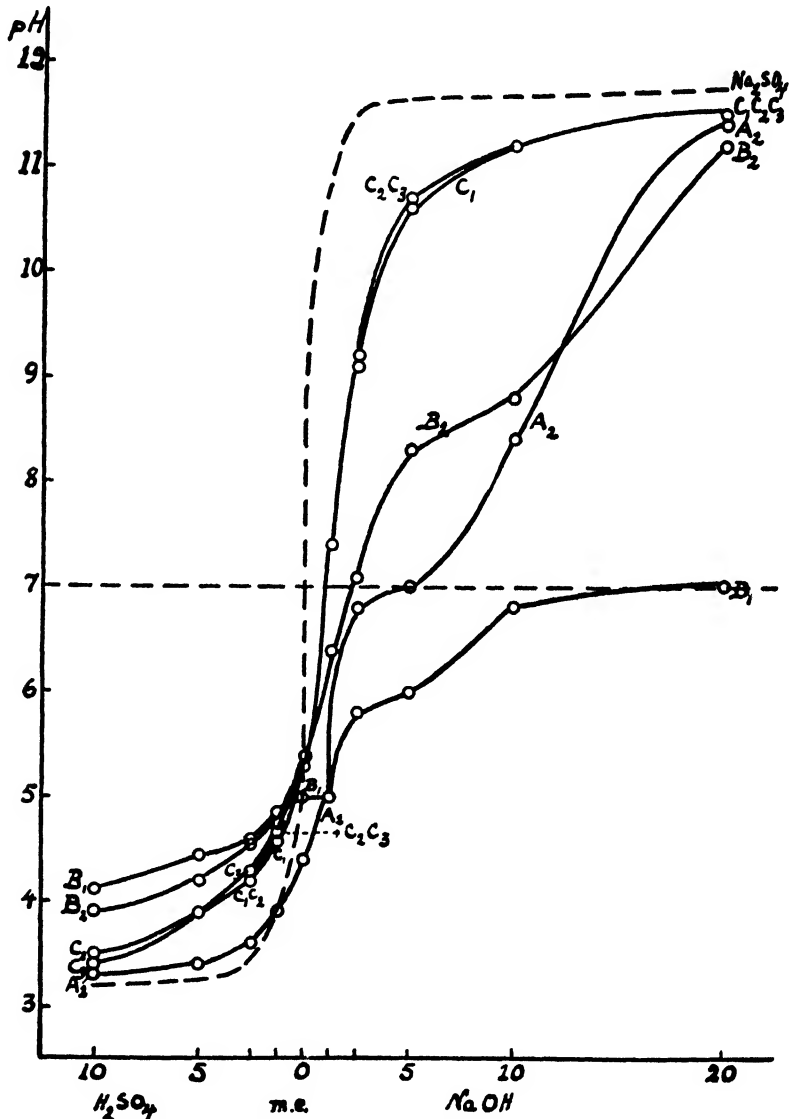


FIG. 10. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTROLYZED SAMPLES FROM THE DIFFERENT HORIZONS OF PROFILE 5 (IRON PODZOL)

tion of the SO<sub>4</sub> ion, which is pronounced when the sesquioxide content is high (13, p. 462). But the salt effect always makes the comparison uncertain.

The combining capacities for bases are also read on the curves. The

quantities depend, as previously stated, on the amounts of colloids present and on their composition. Any linear relationship between the combining

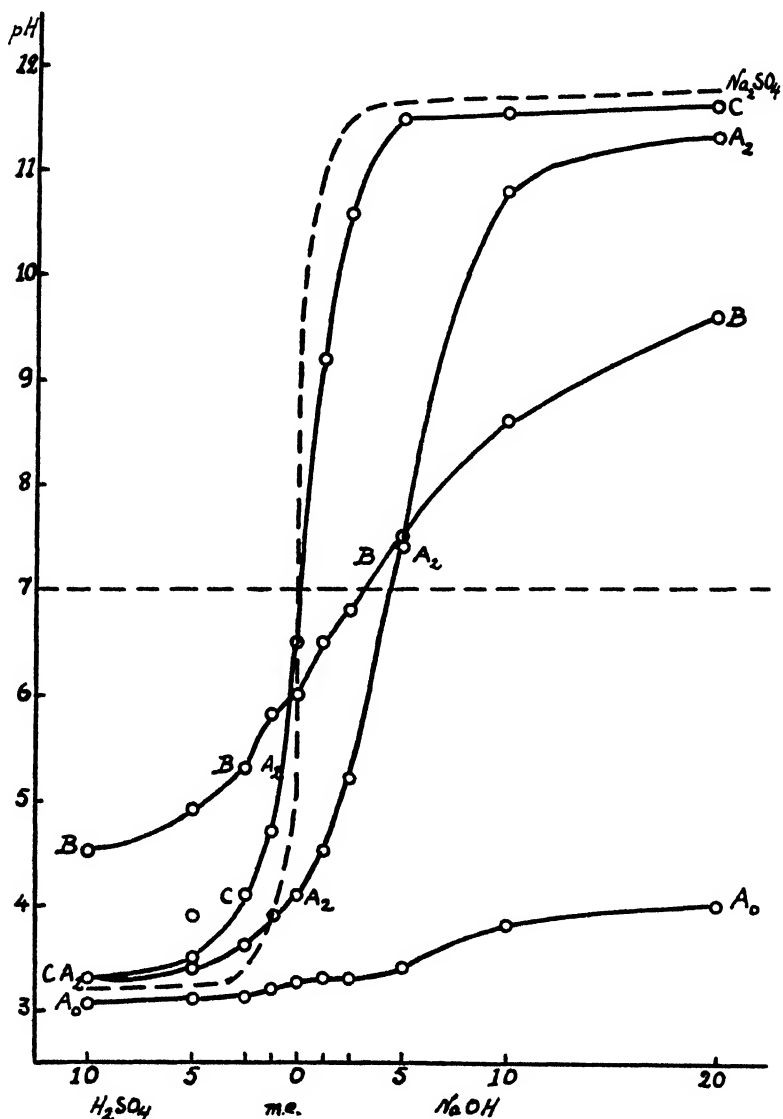


FIG. 11. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF PROFILE 6 (IRON PODZOL)

capacities of the same soil at different pH values is not to be expected. It is entirely possible for a soil to have a very low combining capacity at a comparatively low pH, for instance at the point of neutrality, but this rapidly

increases with the pH because of continuous "liberation" of acid residues which are so weakly acidic that they cannot act as acids except at a high pH. Be-

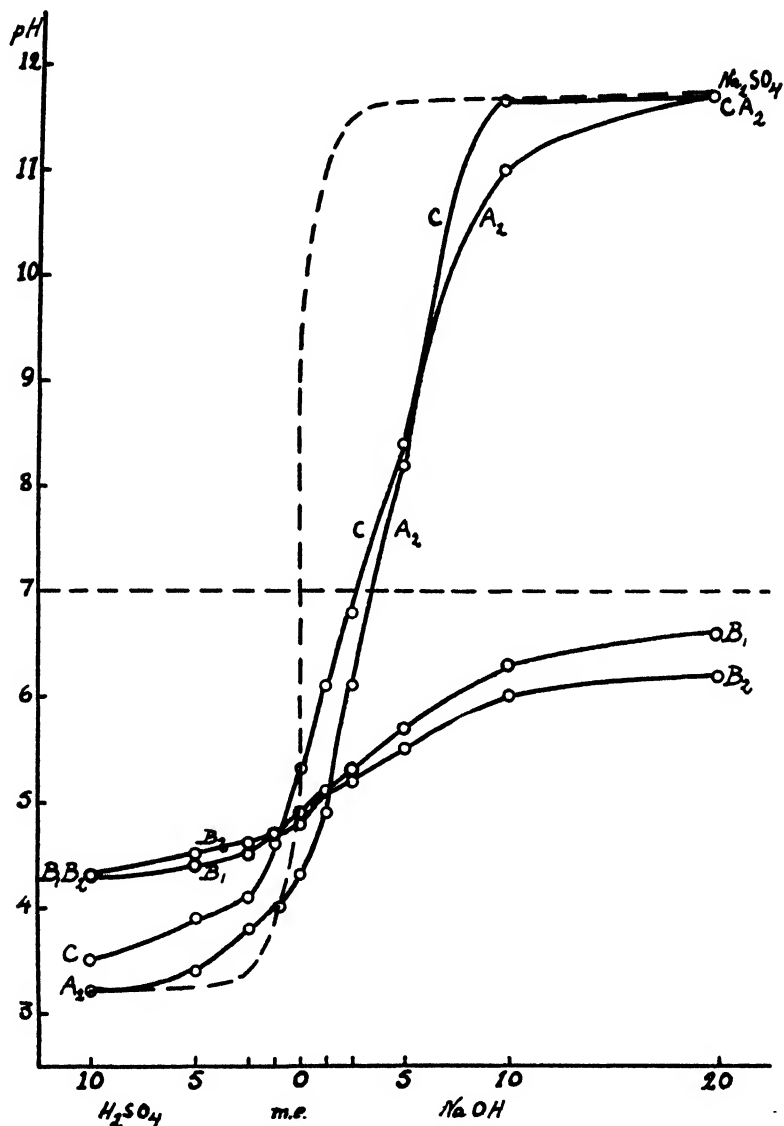


FIG. 12. THE pH OF EXCHANGE NEUTRALITY AND THE CAPACITY TO BIND ACID AND BASE OF ELECTRODIALYZED SAMPLES FROM THE DIFFERENT HORIZONS OF PROFILE 7 (IRON HUMUS PODZOL)

cause of the differences in the composition, the strength of the acid residues, and the different amounts of colloids present, the curves of combining capacity for different soil horizons have very characteristic shapes, as seen from figures



6 to 12. We find a maximum combining capacity in the A-horizon of the brown forest soils and the  $A_0$  layer of the podzols. In the former, a more or less continuous decrease in combining capacity occurs with increasing depth throughout the B and C horizons, whereas the podzols are characterized by a minimum in  $A_2$  and a maximum in the upper part of B. This shows that the amount of colloids is high in this horizon: qualitatively the exchange capacity must be higher in A than in B. The question of why the combining capacities at pH 7 determined by this method do not agree, except in a general way, with the base exchange values determined by leaching has already been answered by Mattson (13, p. 463).

In the case of brown forest soil the curve of the A horizon deviates widely from the curve of the  $Na_2SO_4$  solution. At pH values below the point of exchange neutrality the distance between the curves is fairly small, showing that both the amounts and the strength of the basic residues are rather small. The curves intersect at a fairly low pH, and on the right side of this point, the point of exchange neutrality, the distance between the curves rapidly increases. This means that the colloid complex has a strong acid residue as well as a strong neutralizing power. The latter must be due either to a high colloidal content or to a low acid equivalent weight or to both. The acid equivalent weight of humus is usually much lower than that of the mineral soil colloids. The position of the B curves indicates a different composition of the colloid. Below the point of exchange neutrality the curves lie higher, which, together with the higher pH of exchange neutrality, must mean that the basic residues are stronger than in A. On the other hand the B curves are closer to the  $Na_2SO_4$  curve above the point of exchange neutrality. The organic part of the colloid complex is higher in A than in B, and therefore the qualitative differences in the colloids govern the form of the curves. The form of the C curves shows a lower amount of colloids in C than in B.

The curves for the aclimatic brown forest soil (fig. 8) are of very nearly the same type, except that the curve of the A horizon is still more "flattened." It does not intersect with the line representing pH 7 before a point corresponding to a combining capacity of about 60 m.e. of NaOH per 100 gm. of soil. The point is very uncertain because of the strongly colored solution obtained after the salt solutions are added. This coloration is due to humus and shows a high dispersion or solution.

The podzol curves are very different. The general form of the  $A_0$  curve is to a certain extent like the A curve of the aclimatic brown forest soil (see fig. 11), but there is still a big difference. No intersection with the  $Na_2SO_4$  curve was found; the  $A_0$  curve ran below the  $Na_2SO_4$  curve all the way on the acid side. This would indicate that no basic residues are present, that the colloid complex is not amphoteric, and hence that no point of exchange neutrality exists. From the theoretical standpoint it must be expected that the curves intersect farther out on the acid side, at least they should run together asymptotically. The gradual slope of the curve shows that the intersection

with the pH 7 line lies far above a concentration of 20 m.e. of NaOH per 100 gm. soil. The  $A_2$  curves (see fig. 9-11) approach the  $Na_2SO_4$  curve fairly closely on both sides of the point of exchange neutrality; this is true also for the C curves, whereas the B curves are less steep. The composition of the colloids in  $A_2$  compared to those in B would naturally give less steep  $A_2$  curves. But now the quantities of colloids present in  $A_2$  are very low compared to those in B, as indicated by the oxalate analyses (3, p. 149). Therefore, the qualitative difference is not seen except on the acid side of the point of exchange neutrality. Here the B curves run above the  $A_2$  curves, just as their higher relative content of basic residues requires. But even here the higher content of colloids has its natural influence. The podzol curves are of very much the same type as those obtained by Mattson and Hester from the Lakewood podzol (13, p. 471). The differences in the C curves are certainly due to the higher maturity of this soil: the soil-forming processes are evident even in the deepest part of the profile.

Figure 12, for profile 7, shows the very high amounts of colloids in the B horizons of a profile of the iron humus podzol type. There are high amounts of both acid and basic residues in the colloid complexes as seen from the gradual sloping of the curves on both sides of the fairly high point of exchange neutrality. These facts agree very well with the results of the oxalate analyses previously reported (3, p. 149, 152-153).

Table 6 also gives a comparison between the percentage base saturation in the different horizons of the soils. The figures are calculated from the values read on the curves (fig. 6 to 12) and represent the relation  $\frac{a}{b} \cdot 100$ , where  $a$  is the amount of base combined at the actual pH of the soil and  $b$  the base combined at pH 7. In A of the typical brown forest soil and of the acclimatic brown forest soil we find a high base saturation. The true iron podzols have a very low, or even negative, base saturation in  $A_2$ . The results show a clear difference between podzols and brown forest soils. In respect to base saturation the iron humus podzol seems to take a middle position between podzols and brown forest soils. An interesting case is podzol 2 where all the values are negative. This must mean that all the horizons here are to a certain extent saturated with acids. The same conclusion was previously drawn from the determinations of the ultimate pH.

It is seen that the determinations of the point of exchange neutrality and of the combining capacity of the different soil horizons give very good information about the differences between the two soil types brown forest soils and podzols. The former gives the strength of the acid and basic residues, whereas the latter gives the capacity of the soil to bind acids and bases. Furthermore, the method gives an easy way of determining the degree of base or acid saturation. The results show that the podzols develop through weathering an upper strongly acidoid mineral horizon whereas the brown forest soil profile shows strong acidoid character in the uppermost humus horizon only.

After this paper was written new investigations were published by Mattson and Gustafsson concerning the properties of some Swedish podzols (12). Working with methods similar to those used here they obtained comparable results. There are some differences, however. According to Mattson and Gustafsson the actual pH of the soil solutions in the B and C horizons of podzol profiles is lower than the ultimate pH of their colloidal complexes. As seen from table 6 this is not always the case in the soils here investigated. The following explanation is suggested for this. The actual pH of the soil solution varies somewhat, depending on several circumstances. The soil solution may for some time have a higher or lower pH than the one corresponding to equilibrium conditions with the colloid. The ultimate pH should be a fairly constant quality in each soil horizon, showing the net result of the prevailing weathering. Equilibrium conditions cannot be expected to occur simultaneously with a more or less occasional pH.

#### SUMMARY

The podzol and brown forest soil profiles described in the first part of this paper are further investigated with methods aiming at determination of the amphoteric properties of the soil colloids.

It is shown that base exchange, cataphoresis, ultimate pH, exchange neutrality, and combining capacity give reliable information about the soil forming processes and more or less accurate distinctions between the soil types investigated. Theoretical discussions show that the methods can also be applied for the same purpose in the case of other soil types.

The theory of Mattson of isoelectric weathering is confirmed. The results show that all the soils tested have developed through an acid type of weathering, the podzols being the result of a most acid weathering, the typical brown forest soil originating from a less pronounced acid weathering, and the "aclimatic" brown forest soil taking a middle position between those two soil types, just as could be expected from their occurrence in nature.

Methods such as determinations of the ultimate pH, the pH of exchange neutrality, and the combining capacity at different pH will permit a rapid and conclusive comparative study of the soil forming processes in different soil regions.

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# CHEMICAL CHANGES DURING DECOMPOSITION OF PINEAPPLE TRASH UNDER FIELD CONDITIONS<sup>1</sup>

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In the Hawaiian pineapple fields approximately 100 tons (wet basis) per acre of unproductive old plants are commonly disked down or shredded and plowed under the surface soil after the second ratoon harvest. To a large extent the success of the following crop depends upon the nature and extent of decomposition of this incorporated trash.

Hawaiian pineapple soils have a very low carbon-nitrogen ratio of approximately 7.0 (3) as compared with a ratio of about 10 or more for soils from the mainland United States and other temperate regions. The addition and subsequent decomposition of pineapple trash profoundly alters this ratio. A carbon-nitrogen ratio slightly higher than 7.0 is desirable because of its stimulatory effect on soil microorganisms, which in turn directly influences plant growth. A carbon-nitrogen ratio above 12 to 1 is, on the other hand, undesirable because of the eventual conversion of available nitrogen to the unavailable form under conditions favoring rapid cell multiplication.

Throughout the literature of pedology one can easily locate extensive and comprehensive investigations on the decomposition processes of materials such as rye straw, peat, cottonseed meal, oak leaves, alfalfa, sweet clover, and other plants used for green manures. Starkey (12), in 1924, compiled a rather complete history of the study and progress of the decomposition of organic matter in soils. No effort toward a similar review will be attempted in this report.

Concerning the decomposition of the pineapple plant *Ananas comosus* (L.) Merr. in soil, a recent paper was published by Allen, Abel, and Magistad (1). This investigation was, however, primarily a microbiological approach to the subject and was carried out under artificial laboratory conditions supposedly optimum for the growth of microorganisms. Almost nothing is known of the chemical changes in pineapple trash under actual field conditions.

The primary purpose of the present investigation was to determine under field conditions the rapidity, order, and degree with which the constituents of shredded pineapple plants are changed during decomposition. To attain this

<sup>1</sup> Published with the approval of the director as Technical Paper No. 87 of the Pineapple Experiment Station, University of Hawaii.

<sup>2</sup> Scientific aide and chemist, respectively.

end, samples of soil and trash were obtained from an experimental area at regular intervals and studied chemically in the laboratory.

#### EXPERIMENTAL

An area of approximately one-half acre, located in one of the drier land areas on the island of Oahu, served as the experimental area in the present investigation. This area had been planted in the fall of 1930, and the following crops had been harvested: plant crop in 1932, first ratoon 1933, and second ratoon 1934. On November 1, 1934, a growth on this area of unproductive old pineapple plants approximating 100 tons per acre on the wet basis was shredded. The first weighings and samples for analysis were obtained on the same day, a few hours after shredding. Subsequent samplings were made at 2-week intervals; later samplings were made at 4-week or 6-week intervals as the cycle of decomposition progressed. Altogether 12 samplings were made in a period of 35 weeks.

Four weeks after shredding (November 30, 1934) this area was plowed and disk harrowed. Subsequent plowings were made on February 2, March 29, and May 2, 1935.

#### FIELD METHODS

The most useful and obvious criterion of decomposition in a field experiment is the reduction in total dry weight of the weighable organic matter incorporated into the soil. To obtain this index, field weights of measured sub-areas of trash were made. Weighable trash was arbitrarily taken as all organic material held on a 16-mesh sieve. Sample plots were 0.0003-acre squares. Weights were calculated to tons of trash per acre after corrections for moisture and adhering soil had been made on the basis of iron and aluminum determinations.

Mathematical analyses of previous trial weighings of trash showed that if 10 areas of 0.0003 acre were sampled at one time the resulting standard error would be small enough to give a reliable mean. Hence, all subsequent samplings were made on 10 sub-plots.

Weighings were made by piling the trash contained in the measured area into bamboo baskets. After the total weight was obtained the leaves were separated from the stumps<sup>a</sup> by hand. The stumps were then weighed separately, and the weight of the leaves was obtained by deducting the weight of the stumps from the total trash weight. Approximately one pound of leaves and one pound of stumps from each area were saved, appropriately combined, and taken to the laboratory for analysis. Leaves and stumps were analyzed separately as composites of ten 1-pound sub-samples. The remaining trash gathered from the sub-plots in the process of weighing was returned to the denuded areas after soil samples had been taken.

<sup>a</sup> The term "stump" is used locally to designate the stem of the pineapple plant. This is a massive central storage organ which in the mature plant ranges from 1 to 2 feet in length and 3 to 4 inches in diameter.

Soil samples were obtained through the use of soil augers. Two borings were made in each 0.0003-acre plot. The soil from each pair of plots (1+2, 3+4, etc.) was combined, making five topsoil samples (1 to 12 inches) and five subsoil samples (12 to 20 inches). Care was observed to mix thoroughly all soil samples prior to taking out a definite amount for laboratory analysis.

#### LABORATORY METHODS

Samples of leaves and stumps from the field were first thoroughly chopped up, then dried at 70°C., and this dried material was further subdivided by passage through a Wiley mill. All results are reported on the basis of material dried for 48 hours at 70°C.

Separate samples of both leaves and stumps were used in the analysis for moisture, total nitrogen, and total ash (2). Total iron plus aluminum was also determined (2). The results of the latter determination were employed in developing factors for the correction of adhering soil. Soil analyses showed that soil samples from the experimental area closely approximated 40 per cent iron plus aluminum oxides.

The methods used, with minor adaptations, in the present investigation for the determination of the different organic component parts were those employed by Waksman and Tenney (18) in their study of the composition and decomposition of natural organic materials. These methods were later modified and used by Waksman and Stevens (17) in their studies on the chemical composition of peat. Although the results reveal only the proximate composition and percentages of the various constituent parts, nevertheless they are convenient and useful indications of the chemical changes taking place in the course of decomposition of organic materials in the field.

It is unnecessary to repeat here the clearly presented methods of Waksman and Stevens (17). Because of differences in the nature of the organic material of the present investigation, attention should, however, be called to the following adaptations:

Ten-gram portions of pineapple leaf and stump material were used for analysis.

Soxhlet extractors were used in all three extractions: ether, hot water, and alcohol.

Through experimentation the following extraction periods resulted in comparatively complete extractions: ether 24-26 hours, hot water 16-18 hours, alcohol 4-6 hours.

Hydrolysis of hemicelluloses and celluloses was carried out in 500-cc. Florence flasks using a train of test tube condensers.

Reducing sugars were determined by the methods of Lane-Eynon (2) and Munson-Walker (2).

Total available nitrogen (ammonia plus nitrate) in soils was extracted by Harper's method (4) using 10 per cent NaCl and distilling with Devardo's alloy for nitrate reduction. This determination was made as soon after sampling as time permitted. Soil samples were then air-dried. Total nitrogen was determined on the air-dry soil by the method of Gunning and Hibbard (2); organic matter, by the wet oxidation method (5); available potassium,



by the method of Volk and Truog (16); and easily soluble phosphorus, by the method of Truog (15). Moisture determinations (2) were made and all results calculated on the dry soil basis.

# DISCUSSION OF RESULTS

## *Dry weights*

The dry weights of the pineapple trash at regular intervals throughout the decomposition period of 35 weeks are presented in table 1.

On November 1, 1934, at the time of shredding, the first weighings of trash were obtained. Means of ten 0.0003-acre areas showed the leaf fraction of the trash to be present at the rate of 18.6 tons per acre on the dry basis. The stumps had an average weight of 8.4 tons per acre, dry basis. This is a total trash weight of 27.0 tons per acre on the dry basis. Moisture determinations averaged 70 per cent for total trash. When a standard error of  $\pm 3.84$  is

TABLE 1

*Trash weights at various stages of decomposition in tons per acre—dry basis*  
(Corrected for adhering soil)

MATERIAL	WEEKS											
	0	2	5	7	9	11	13	15	19	23	29	35
Leaves.....	18.6	18.0	14.3	22.3	18.6	18.8	7.6	6.3	3.7	2.8	0.9	1.6
Stumps.....	8.4	8.2	6.1	5.5	5.2	7.5	4.0	4.0	1.7	1.8	1.6	1.6
Total trash.....	27.0	26.2	20.4	27.8	23.8	26.3	11.6	10.3	5.4	4.6	2.5	3.2

taken into consideration, it can be concluded that the average trash weight, on the wet basis, in this experimental area approximated 100 tons per acre. This is good average tonnage for other pineapple areas.

At the end of the 35-week decomposition period the following weights on the dry basis were obtained: leaf material, 1.6 tons; stump material, 1.6 tons; total trash, 3.2 tons, per acre. Calculations involving the weights obtained at the start of the experiment and those obtained at the end of 35 weeks show that 91 per cent of the leaf material and 81 per cent of the stump material had been decomposed.

Magistad (unpublished data) was able to obtain complete decomposition of finely chopped pineapple stems in 3 months in soils kept at 30 per cent moisture content under laboratory conditions. Under field conditions such a rapid rate of decomposition is impossible because of three important uncontrolled factors:

Incomplete mechanical disintegration of the pineapple plants during the process of shredding. Sometimes whole plants are merely uprooted and left on the surface to decompose.

Wide variation in the moisture supply, which is often so low as greatly to retard decomposition.

The available nitrogen supply varies widely between regions in which pineapple is grown.

At the end of the 35-week period it became evident that further decrease in weights would be extremely meager over an extended period. At this stage approximately 90 per cent of the total trash, both leaf and stump material, was made up of resistant hemicelluloses, celluloses, lignins, and crude protein. Such material, still in a slow process of decomposition, gradually approaches the final product commonly called soil "humus." With deep plowing this heterogeneous mass becomes an important agent in the physical improvement of pineapple areas low in organic matter.

From table 1 it is surprising to find that the trash weights obtained during the fourth, fifth, and sixth samplings were unusually high. It was discovered that this was due to an error in the sampling method. In the first six samplings, plots had been selected at various areas in the field most likely to give an average weight. This was not a truly random method of sampling, since personal judgment was a deciding factor. This method was revised, and from then on trash weights showed a consistent decrease as decomposition proceeded. In the revised method the field was laid out in a Latin square arrangement with provisions for 100 sampling plots. Selection of 10 plots for each of 10 samplings was then determined by the casting of dice.

#### ORGANIC FRACTIONS

Proximate chemical analyses of the various organic fractions in pineapple trash showed that the quantitative changes of the constituent fractions throughout the decomposition period of 35 weeks agreed fairly well with those reported by Tenney and Waksman (14) in their studies on mature corn stalks, rye straw, mature oak leaves, and mature alfalfa plants. Table 2 summarizes the total losses in per cent and in tons per acre of the various fractions for the decomposition period of 35 weeks.

*Ether-soluble fraction.* The ether-soluble fraction, comprising the fatty oils, nitrogenous fats, and parts of the wax-like and resin-like substances, showed a steady decrease throughout the period of decomposition. At the end of 35 weeks, 97.1 per cent of the original 0.69 tons per acre in the leaf material was no longer accounted for in the material held on a 16-mesh sieve. In the stump material 93.3 per cent had been decomposed.

*Hot-water-soluble fraction.* In both the leaf material and stump material this fraction along with the ether-soluble fraction showed the greatest per cent loss during the 35-week decomposition period. This loss in the water-soluble fraction is not surprising, since it has been shown time and again by numerous investigators that this fraction, containing the carbon compounds most easily available to microorganisms, always disappears readily and in the largest amounts.

Analyses for water-soluble reducing sugars showed an initial concentration in the leaf material of 5.94 per cent of the dry material. In 2 weeks this amount was reduced by over a half, 2.61 per cent remaining. In 5 weeks it had been reduced to a fraction of a per cent, and in 7 weeks only traces of re-

ducing sugars were detectable. This loss was almost identical in the case of stump material.

Water-soluble nitrogen showed a continuous drop from 0.26 to 0.11 per cent in the leaf material and from 0.32 to 0.14 per cent in the case of the stump material during the first 23 weeks. Thereafter an increase was detected, the leaf material showing a final percentage of 0.22 and the stump material a percentage of 0.24 at the end of 35 weeks. This increase in available nitrogen toward the end of the decomposition period can most probably be explained by the fact that most of the easily available carbon had been used up, thereby resulting in a reduction or limitation of the activities of the microorganisms carrying on the decomposition process. Death of the microorganisms was

TABLE 2

*Losses in the various chemical fractions of pineapple trash due to a 35-week decomposition period*

CHEMICAL CONSTITUENTS	LEAF MATERIAL						STUMP MATERIAL					
	Prior to decomposition, 18.6 tons/acre, dry		After 35 weeks, 1.6 tons/acre, dry		Loss		Prior to decomposition, 8.4 tons/acre, dry		After 35 weeks, 1.6 tons/acre, dry		Loss	
	Per cent composition	Tons/acre, dry basis	Per cent composition	Tons/acre, dry basis	Per cent loss	Tons/acre, loss	Per cent composition	Tons/acre, dry basis	Per cent composition	Tons/acre, dry basis	Per cent loss	Tons/acre, loss
Ether-soluble fraction.....	3.72	0.69	1.14	0.02	97.1	0.67	1.82	0.15	0.52	0.01	93.3	0.14
Hot-water-soluble fraction.....	27.13	5.05	8.75	0.14	97.2	4.91	23.14	1.94	8.03	0.13	93.3	1.81
Alcohol-soluble fraction.....	2.06	0.38	2.77	0.04	89.5	0.34	2.95	0.25	1.99	0.03	88.0	0.22
Hemicelluloses.....	19.08	3.55	22.54	0.36	89.9	3.19	29.54	2.48	20.11	0.32	87.1	2.16
Celluloses.....	24.92	4.64	38.30	0.61	86.9	4.03	16.88	1.42	35.48	0.57	59.9	0.85
Ash-free lignin.....	7.64	1.42	25.41	0.41	71.1	1.01	7.54	0.63	29.63	0.47	25.4	0.16
Crude protein.....	2.64	0.49	4.36	0.07	85.7	0.42	2.53	0.21	3.90	0.06	71.4	0.15
Total ash.....	5.90	1.10	9.09	0.15	86.4	0.95	3.19	0.27	7.22	0.12	55.6	0.15

accelerated, followed by disintegration of cell protein and subsequent release of available nitrogen. It is also true that the reduced carbon-nitrogen ratio at this stage encouraged an accumulation of nitrate. This was substantiated analytically by a corresponding reduction in crude proteins during the period of increasing available nitrogen. The foregoing results are in agreement with those of Martin (7) and Sturgis (13), who in decomposition studies have shown that nitrate accumulates along with the formation of soil humus.

*Alcohol-soluble fraction.* Although the percentage of this fraction at no time throughout the decomposition period showed a great variation, yet, when weights are considered, an evident loss can be shown to have occurred. No doubt the greater part of this loss was due to the destruction of the higher alcohols. The more resistant waxes, resins, tannins, and bitter substances

can be expected to make up the major portion of this fraction at the end of the 35-week period.

*Hemicelluloses.* Allen, Abel, and Magistad (1) found that under laboratory conditions hemicelluloses in chopped pineapple stumps and leaves showed a surprisingly low degree of decomposition although it was shown that a large percentage of the microflora of the soil was able to utilize this substance. Under field conditions it seems that a considerable loss occurs. The percentage of hemicellulose persisting in the trash at any given period throughout the cycle fluctuated consistently at approximately 20 per cent. This shows that the rate of decomposition of these compounds was in a fair proportion to the rate of decomposition of the total trash.

When the decomposition of hemicelluloses was computed on the basis of reduction in tons per acre, at the conclusion of the 35-week period, there was a loss of 89.5 per cent of the original 3.55 tons present in the leaf material. In the stump material a loss of 87.1 per cent was recorded. Tenney and Waksman (14) noted the following percentages of hemicelluloses remaining in various materials after a decomposition period: corn stalks, 12.35 per cent of the original after 405 days; rye straw, 53.22 per cent after 386 days; mature oak leaves, 54.91 per cent after 386 days; and alfalfa, 37.09 per cent after 405 days. Pineapple leaves and stumps compare favorably with corn stalks in that 10.1 and 12.9 per cent, respectively, remained after a somewhat shorter decomposition period of 245 days.

*Cellulose.* Cellulose is known to undergo decomposition to a fairly large extent under natural conditions, both aerobically and anaerobically. It is generally concluded, however, that the decomposition usually requires a greater length of time as compared to the rate of decomposition of the other plant constituents. This is especially true if it is combined with the most resistant of all plant substances, the lignins, to form ligno-celluloses.

In the present investigation 13.1 per cent of the original total cellulose in the leaf material remained after 35 weeks. In the stump material a far greater amount remained undecomposed, 40.1 per cent of the original 1.42 tons being detectable after 35 weeks. It is obvious that a far greater fraction of the cellulose in the stump material is in a more resistant form than that found in the leaf material.

*Lignins.* It has been generally concluded by the majority of investigators and has been proved that the most resistant fraction of natural organic materials is the lignins combined as ligno-proteins and ligno-celluloses. Pineapple trash conforms to this established contention.

Consistently rising percentages of lignin for both pineapple leaf and stump material definitely prove that the decomposition of this fraction did not keep pace with the decomposition of the other organic fractions or with trash taken as a whole. The original analysis showed an initial lignin concentration of 7.64 per cent in the leaf material and 7.54 per cent in the stump material. At the end of 35 weeks the concentration of lignin on the percentage basis had

increased to 25.41 per cent in the leaf material and 29.63 per cent in the stump material. Calculated on the dry weight basis per acre, however, the lignin showed a considerable reduction.

The decomposition of lignin was much faster in the leaf material than in the stump material. In the former case, after 35 weeks, only 28.9 per cent of the original amount remained, whereas in the case of the stump material 74.6 per cent was accounted for. From the residual lignin angle, pineapple leaf material compares favorably with corn stalks, and pineapple stump material is much akin to mature oak leaves (14).

The vast differences in the rate of decomposition of lignin between the two types of pineapple material can be explained by their differences in physical structure. The fibrous tissues, including nearly all of the lignins found in the pineapple leaf exist as thin strands which are easily attacked. In the stump material the fibrous tissues are much heavier in structure. The former physical type lends itself much more easily to mechanical disintegration, which in the present study is an accountable factor.

*Crude protein.* The percentage of crude protein rose steadily as decomposition proceeded, the sharpest rise occurring during the first 5 weeks. Toward the end of the 35-week period, crude protein decreased slightly. Available nitrogen showed a reciprocal fluctuation, being reduced at the beginning and increasing toward the end of the decomposition period.

This rise and fall of both available nitrogen and crude protein can easily be explained from the microbiological standpoint, as previously referred to in the discussion of water-soluble nitrogen.

#### SOIL

In any field decomposition study of organic materials, soil analyses of the experimental areas are indispensable because of the intimate relation between material and soil. Table 3 summarizes the results of soil analyses performed in the present study. Among the most important determinations is the quantitative relationship of total carbon to total nitrogen. In any soil undisturbed for a long time, a definite equilibrium between carbon and nitrogen tends to be established. Cultivation, with the addition of carbonaceous or nitrogenous materials, changes this ratio. Reversion to the normal ratio is a constant process (11). Other important quantitative determinations are those of total available nitrogen, available potassium, and easily soluble phosphorus.

*Carbon-nitrogen ratio.* Dean (3) in a summary of 223 Hawaiian pineapple soils has shown that the average carbon-nitrogen ratio under local climatic conditions is very near 7 to 1. The organic matter content was analyzed by the method of Rather (8, 9), which probably gave too high results due to incomplete removal of the hydrated colloids of the soil.

Using the wet combustion method (5) to determine the organic matter in



85 Hawaiian pineapple soils, Magistad (unpublished data) obtained a carbon-nitrogen ratio lower than 7 to 1. A summary of Magistad's results show:

FIELD NUMBER	PER CENT TOTAL ORGANIC MATTER	PER CENT TOTAL NITROGEN	C:N RATIO
1 Kunia	2.86	0.280	5.9
15 Kunia	2.55	0.267	5.5
19 Kunia	2.84	0.281	5.9
302 Molokai	1.58	0.187	4.9
321 Molokai	1.74	0.196	5.4

In the present investigation, a very low carbon-nitrogen ratio of 4.8 to 1 was obtained prior to the incorporation of the pineapple trash (table 3). With the shredding of 27 tons (dry basis) of trash, approximately 58 per cent of which is carbon, the carbon-nitrogen ratio was raised to 10.8 (fig. 1). Approximately 360 pounds of nitrogen was contained in the 27 tons of trash. This was taken into consideration in the carbon-nitrogen ratio calculations.

At the end of the 35-week decomposition period the carbon-nitrogen ratio resulting from the analysis of 16-mesh soil sampled to a depth of 1 foot was 5.2. The calculated carbon-nitrogen ratio, which takes into consideration the undecomposed residue of the incorporated trash, was 5.9; this ratio had dropped from 10.8 in the 35 weeks (fig. 1).

The difference between the analyzed ratio of 5.2 and the calculated ratio of 5.9 is attributed to the resistant fractions of trash which withstood decomposition in the 35-week period; namely, the ligno-celluloses, the ligno-proteins, and some hemicelluloses. This residue in a slow and relatively constant rate of chemical change will finally approach the chemical state of soil "humus." Resisting further decomposition, this heterogeneous mass becomes an important factor in the physical improvement of our pineapple soils low in organic matter.

*Available nitrogen.* Throughout the 35 weeks of decomposition, available nitrogen in the surface foot of soil never dropped below 20 p.p.m. (fig. 2). This was to be expected because of the very low carbon-nitrogen ratio and despite the fact that it was raised to 10.8 with the addition of trash. Russell (10) places the critical carbon-nitrogen ratio, below which nitrate accumulates, at 12 to 1.

Decrease in the available nitrogen of the topsoil with an increase in the subsoil during the thirteenth, fifteenth, nineteenth, and twenty-third weeks was the result of heavy spring rains. At one time (twenty-third week) the available nitrogen of the subsoil was actually greater than that of the topsoil. Available nitrogen in the topsoil showed a constant increase during the dry summer months.

*Replaceable potassium.* An interesting result of the soil analysis was the continual increase in replaceable potassium in the soil throughout the decom-

position period of 35 weeks (table 3). Replaceable potassium actually increased from 0.31 to 1.03 m.e.  $K_2O$ /100 gm. soil, or from 350 to 1160 pounds

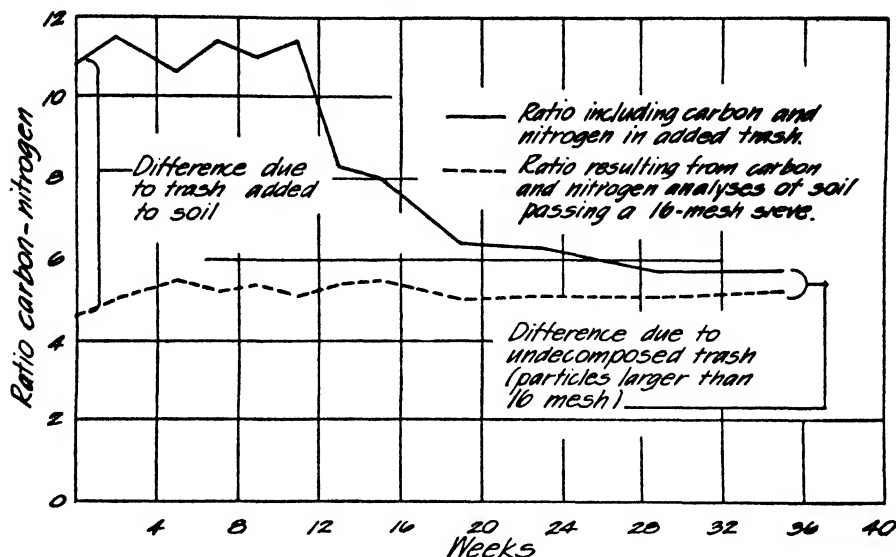


FIG. 1. SOIL CARBON-NITROGEN RATIOS DURING A 35-WEEK DECOMPOSITION PERIOD OF PINEAPPLE TRASH

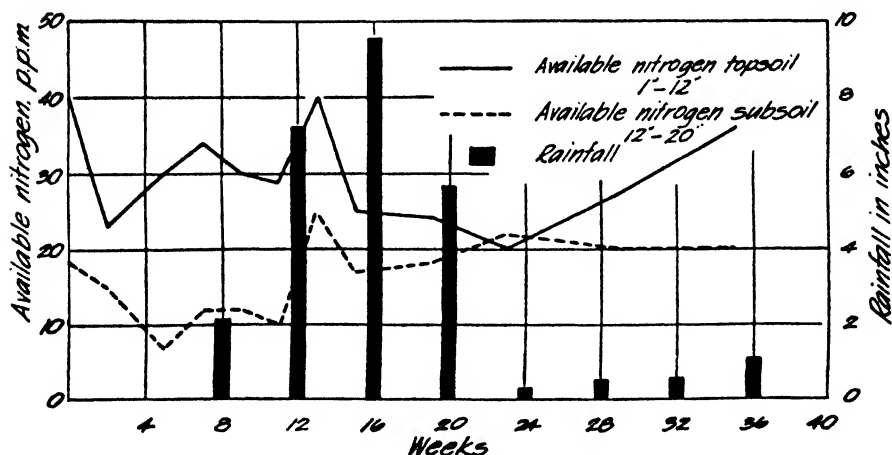


FIG. 2. AVAILABLE NITROGEN OF SOILS DURING A 35-WEEK DECOMPOSITION PERIOD OF PINEAPPLE TRASH  
(P.p.m., dry basis)

per acre foot of soil on the basis of 2,400,000 pounds of soil per acre foot. This increase was undoubtedly due to the release of replaceable potassium from the decomposing material. According to Magistad (6) probably no



response by pineapple plants would result from potassium fertilization of a soil containing over 0.5 m.e.  $K_2O$ /100 gm. soil.

*Easily soluble phosphorus.* The presence of this element in the soil showed a steady decrease during the first 13 weeks of decomposition (table 3). This was probably due to the utilization of this element by the microorganisms during their most active period of decomposition. Toward the end of the cycle, easily soluble phosphorus returned to its original level.

#### SUMMARY

To determine under field conditions the rapidity, order, and degree with which the constituents of shredded pineapple plants are changed during decomposition, approximately one-half acre of old pineapple plants with a top growth of 100 tons per acre (wet basis) was tractor-shredded on November 1, 1934, and allowed to decompose for a period of 35 weeks. It should be pointed out clearly that this period includes the months generally highest in rainfall and that, therefore, the organic material studied in the present experiment received the benefit of a large amount of necessary moisture during the early stages of its decomposition. Application of the results of the present study to decomposition periods involving the drier months of the year may lead to serious errors.

At regular intervals during the decomposition period leaf, stump, and soil samples were chemically analyzed. The term "stump" is used locally to designate the stem of the pineapple plant—a massive central storage organ which in the mature plant ranges from 1 to 2 feet in length and 3 to 4 inches in diameter.

At the end of the 35-week decomposition period the residual leaf material was only 9 per cent of the original 18.6 tons per acre dry leaf portion of the trash. Of the original 8.4 tons of stumps (dry basis), 19 per cent remained undecomposed.

All of the organic fractions found in trash underwent decomposition to a certain degree, some proceeding at a greater rate than others. In the order of increasing resistance to decomposition, the various fractions were as follows: water-soluble fraction, ether-soluble fraction, alcohol-soluble fraction, hemicelluloses, celluloses, and lignins.

Water-soluble reducing sugars were decreased from 5.94 per cent to 2.61 per cent in the leaf material during the first 2 weeks of decomposition. At the end of 7 weeks only traces of reducing sugars were detectable. This loss was also shown to be true in the case of the stump material.

Crude protein in trash increased as decomposition proceeded, whereas water-soluble nitrogen decreased. During the last 12 weeks a decrease in crude protein was detected with a corresponding increase in water-soluble nitrogen.

The original soil carbon-nitrogen ratio was 4.8 to 1. With the addition of 27 tons of trash (dry basis), approximately 58 per cent of which was carbon, the ratio was increased to 10.8. At the end of 35 weeks of decomposition the carbon-nitrogen ratio was reduced to 5.9, 3.2 tons of residual trash held on a 16-mesh sieve being taken into consideration. The carbon-nitrogen ratio of 16-mesh soil was 5.2.

Recent carbon-nitrogen ratio studies including the present investigation tend to show that the ratio in certain Hawaiian soils is even narrower than the 7 to 1 ratio reported by Dean (3) in 1930. A ratio of 5 or 6 to 1 seems to be a closer approximation for the drier land areas reported in this paper.

Available nitrogen in soils decreased during the first 23 weeks of decomposition but increased during the last 12 weeks.

Replaceable potassium in soil continually increased from 0.31 to 1.03 m.e.  $K_2O/100$  gm. soil, or from 350 to 1160 pounds per acre foot, during the 35-week period. This was released from the incorporated trash during decomposition.

Easily soluble phosphorus decreased during the earlier stages of decomposition, regaining its former level in the soil toward the end of the decomposition period.

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# ZINC CONTENT OF SOILS IN RELATION TO PECAN ROSETTE

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In recent years zinc has been found to be an essential element for normal plant growth. It is not required in large amounts, however, and some plants or trees either require less of the element or are better able than others to obtain their needs from the same soil environment. The pecan is rather sensitive to a deficiency of zinc due either to insufficient amounts present in the soil or to its unavailability; this deficiency results in a condition of the tree known as "pecan rosette."

Several investigators (1, 2, 3, 4, 5) have controlled pecan rosette by using zinc salts, usually the sulfate, in one of three ways; namely, as a spray applied to the foliage, as an injection into the trunk, or as an application to the soil. The spray application is equally effective on any soil type. With the limited data available it would seem that trunk injections, when sufficient amounts are applied, are also equally effective on any soil type. On the other hand, soil applications in certain amounts are effective on some soil types, whereas on others the same or larger amounts have no effect.

Pecan rosette has not been observed on some soil types, whereas on other soil types rosette, unless controlled, is a limiting factor in pecan development. In Louisiana and Texas, pecan rosette is more prevalent on basic soils than on acid soils. All of the zinc present in soils may or may not be available to the trees. The availability of the zinc may depend on the chemical composition of the soil and possibly on its reaction. It is reasonable to expect that the amount present in the various soils might have something to do with supplying the tree's need for the element. This paper deals with the zinc content of several soils, soil reaction, and the prevalence of rosette.

Details of the method developed for determining the zinc content of the soils are to appear in another article. In brief, it consists of fusing the soil with potassium pyrosulfate and disintegrating the fusion in hot hydrochloric acid. The silica is removed by filtration, and the washings are evaporated. The acidity of the solution is fixed at 0.4 *N*, and the interfering members of the first hydrogen sulfide group are removed. The solution is buffered, a filter aid added, and the pH fixed at about 3.5. The zinc is precipitated with hydrogen sulfide, filtered, and dissolved with 1 *N* hydrochloric acid. The zinc in the acid solution is determined nephelometrically by the ferrocyanide method.

The total zinc content and the reaction of 32 soil profiles have been determined. All of these data are not presented here as they are not needed to bring out the points involved. The zinc content of the soil and the reaction were determined for depths varying from 3 to 12 feet, with most determinations made to a depth of 6 feet. On cultivated soils the soil samples were taken at intervals of 0-6, 6-12, 12-24, 24-36 inches, etc.

The total zinc content varies considerably in the soils used in the determinations. The highest amount found in an acre 6 inches was 146 pounds, and the smallest amount, 8 pounds. These figures for the zinc content and the ones used in the tables are computed on the basis of a weight of two million pounds for an acre 6 inches of soil.

The zinc content and the soil reaction of six calcareous pecan soils from Texas are given in table 1. The smallest amount of zinc in these basic soils

TABLE 1  
*Pounds of zinc per acre and reaction of six calcareous soils*

DEPTH	CATALPA SILTY CLAY LOAM, BROWNWOOD, TEX.		FRIO SILTY CLAY LOAM, UVALDE, TEX.		CATALPA SILTY CLAY LOAM, CIRCLEVILLE, TEX.		CATALPA CLAY LOAM, GEORGE- TOWN, TEX.		CATALPA SILTY CLAY LOAM, LOW PHASE SECOND BOT- TOM, LULING, TEX.		CATALPA SILTY CLAY LOAM, THIRD BOTTOM, LULING, TEX.	
	Zinc	pH	Zinc	pH	Zinc	pH	Zinc	pH	Zinc	pH	Zinc	pH
<i>inches</i>	<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>	
0-6	126	8.10	106	7.70	76	7.40	146	8.00	106	7.55	86	8.40
6-12	126	8.20	106	7.70	66	7.40	146	7.70	106	7.40	86	8.40
12-24	212	8.25	212	7.23	232	7.55	252	7.77	252	7.15	152	8.40
24-36	292	8.35	252	7.20	212	7.40	252	7.70	252	7.52	172	8.30
36-48	212	8.40	252	7.80	...	...	...	...	...	...	...	...
48-60	232	8.55	252	7.75	...	...	...	...	...	...	...	...
60-72	232	8.50	252	8.10	...	...	...	...	...	...	...	...
Total to 36...	756	....	676	....	586	....	796	....	716	....	496	....

for any one 6-inch soil horizon is 66 pounds, and the largest amount is 146 pounds. The total amount of zinc in an acre from the surface down to 36 inches varies from 496 to 796 pounds. The soil reaction is above pH 7 for all the horizons sampled. Pecan trees have rosetted severely in all the locations where the soil samples were taken except one which is of special interest: when these soil samples were taken pecan trees were not rosetting in the second bottom on the Luling, Texas, farm. All the pecan trees planted on the third bottom were rosetting severely. Table 1 shows that the amount of zinc is considerably lower and the soil reaction is higher in the third bottom than in the second.

The zinc content per acre 6 inches and the soil reaction for six soils with both acid and basic horizons are given in table 2. The smallest amount of zinc is 8 pounds, and the largest is 126 pounds for an acre 6 inches of soil.

The total zinc content for an acre to a depth of 36 inches varies from 112 to 756 pounds. The soil reaction for the horizons varies from pH 5.75 to pH 9.08. The rosette condition of pecan trees varies considerably in the orchards from which the soil samples given in table 2 were taken. The writers have never observed any rosette in the orchard from which the Monroe silt loam sample

TABLE 2

*Pounds of zinc per acre and reaction of soils with both acid and basic horizons*

DEPTH	MONROE SILT LOAM, MONROE, LA.		ACADIA VERY FINE SANDY LOAM, HOUSTON, TEX.		WINDTHORST FINE SANDY LOAM, CALCAREOUS PHASE, STEPHENVILLE, TEX.		WINDTHORST SANDY LOAM, RISING STAR, TEXAS		PLEDGER SILTY CLAY LOAM, BRAZORRA, TEXAS		YABOLLA LOAM, SHREVEPORT, LA.	
	Zinc	pH	Zinc	pH	Zinc	pH	Zinc	pH	Zinc	pH	Zinc	pH
<i>inches</i>	<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>	
0-6	86	6.10	8	6.45	18	7.70	18	7.05	126	7.00	50	7.50
6-12	86	5.75	18	6.30	22	7.75	22	7.00	126	6.92	50	7.46
12-24	172	5.75	68	6.60	84	6.80	36	6.00	252	7.64	92	7.35
24-36	192	7.47	84	7.00	116	6.50	36	5.90	252	7.87	60	7.00
36-48	172	8.35	76	7.25	52	8.10	...	...	252	7.85	60	6.85
48-60	132	9.07	116	7.67	...	...	...	...	212	8.00	100	6.55
60-72	172	9.08	124	8.00	...	...	...	...	212	8.15	52	6.50
Total to 36...	536	....	178	....	240	....	112	...	756	....	252	....

TABLE 3

*Pounds of zinc per acre and reaction of some acid soils*

DEPTH	OCKLOCKONEE FINE SANDY LOAM, TOLEDO, TEX.		RUSTON FINE SANDY LOAM, CADDO PARISH, LA.		NORFOLK FINE SANDY LOAM, CADDO PARISH, LA.		NORFOLK FINE SANDY LOAM, WINONA, TEXAS	
	Zinc	pH	Zinc	pH	Zinc	pH	Zinc	pH
<i>inches</i>	<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>		<i>lbs.</i>	
0-6	66	6.00	26	6.15	18	6.10	18	5.58
6-12	66	5.10	24	5.95	18	5.60	14	5.60
12-24	132	5.15	68	5.45	36	5.20	52	5.00
24-36	132	4.88	84	5.52	44	5.40	52	5.00
36-48	132	4.35	100	5.25	...	....	52	5.10
48-60	132	4.25	100	5.20	...	....	52	5.60
60-72	...	....	...	....	...	....	52	5.20
Total to 36...	396	....	202	....	116	....	136	....

was taken. This soil has a relatively high zinc content and an acid reaction of the soil to a depth of 24 inches at least. The orchards on all the other soils given in the table have rosette in varying degree of severity, but none of them have rosette as severely as the orchards from which the soil samples given in table I were taken. Rosette is not particularly severe in the orchard which has the lowest zinc content. The soil reaction is only slightly alkaline in the

surface horizon, neutral in the 6-12 inch horizon, and basic in the 12-36 inch horizon.

The zinc content per acre 6 inches and the soil reaction for four acid soils are given in table 3. The zinc content varies from 14 to 66 pounds per acre 6 inches and from 116 to 396 pounds for an acre to a depth of 36 inches. The soil reaction for the horizons varies from pH 4.25 to pH 6.15. No rosette has been observed in the pecan orchard on the Ochlockonee fine sandy loam from which the soil sample was taken. The zinc content of 396 pounds for an acre 0-36 inch horizon is almost half of the amount present in most of the basic soils shown in table 1. The Ruston fine sandy loam soil has a zinc content of 202 pounds per acre for the 0-36 inch horizon. The whole profile tested is decidedly acid. Pecan trees are not rosetting in this part of the orchard. The zinc content in this profile is less than a third of that of most of the soil profiles shown in table 1. The two Norfolk soils have a very low zinc content. The soil reaction is acid for both profiles. In the parts of the orchards from which these samples were taken pecan trees are rosetting.

The results given in this paper show that the zinc content of the basic soils examined is generally higher than the zinc content of the acid soils and that even though the basic soils contain appreciable quantities of zinc there is considerable rosette in pecan trees growing on these soils, indicating that the zinc is unavailable. On soils having both acid and basic horizons the trees, as indicated by rosette condition, seem to be favorably influenced by the acid horizons. Soils having all acid horizons support pecan trees relatively free of rosette when the soil contains a moderate quantity of zinc, indicating that this zinc is available. Acid soils containing very small quantities of zinc produce trees which rosette, indicating that the very small quantities of zinc which they contain, though available, are not sufficient for normal functioning of pecan trees. In studying the functions of zinc in the control of pecan rosette, it is indicated that the zinc content of the soil, as well as the chemical composition and the reaction of the soil, must be taken into consideration.

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# THE RESPONSE OF RHIZOBIA TO NATURAL HUMIC ACID

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The results of studies (1, 2, 8) at this laboratory, confirmed recently by other investigators (11, 12), have shown that certain strains of legume nodule bacteria are unable to make an appreciable growth in an ordinary synthetic medium, prepared from pure chemicals, but require an additional substance previously designated as "coenzyme R." This material, which is essential for respiration and growth, is found widely distributed in plant and animal materials.

Other investigations (6, 7, 9, 10) with *Azotobacter chroococcum* and *Azotobacter vinelandii*, also carried out at this laboratory, have shown that the growth of these organisms is commonly increased from two- to five-fold by the addition of natural humic acid extracted from soil. This stimulation is due in large part to the iron which is present in such form that it is not readily precipitated.

These two lines of research suggest, therefore, that the growth stimulation produced by natural humic acid on rhizobia may be due not only to its content of the essential growth factor, but, in addition, to its available iron. The work reported in this paper was undertaken with the aim of learning more about the latter phase of the problem.

## METHODS

The cultural medium, the same as that used previously (1), consisted of inorganic salts (without added iron), 1 per cent sucrose or dextrose, and 5 mgm. N ( $\text{KNO}_3$ ,  $\text{NaNO}_3$ , or  $\text{NH}_4\text{NO}_3$ ) per culture of 25 cc. The data reported in tables 1-4 were obtained by growing the organisms at 28°C. in 250-cc. Erlenmeyer flasks and making counts at intervals by means of a haemocytometer modified for bacterial counts. The results shown in figure 1 were obtained by the usual Warburg respiration technique. In this case the organisms were first grown in Erlenmeyer flasks, centrifuged under aseptic conditions, and 800 millions in 4 cc. of medium added to each sterilized Warburg vessel.

Natural humic acid was prepared (5) from a rich garden soil by extraction with alkali. Repeated precipitation with acid and dissolving in alkali mark-

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edly reduces the coenzyme R content of the preparation. In the preparation of synthetic humic acid, described in detail elsewhere (10), sugar is boiled in strong  $H_2SO_4$ , the mixture centrifuged, and the solid phase extracted with

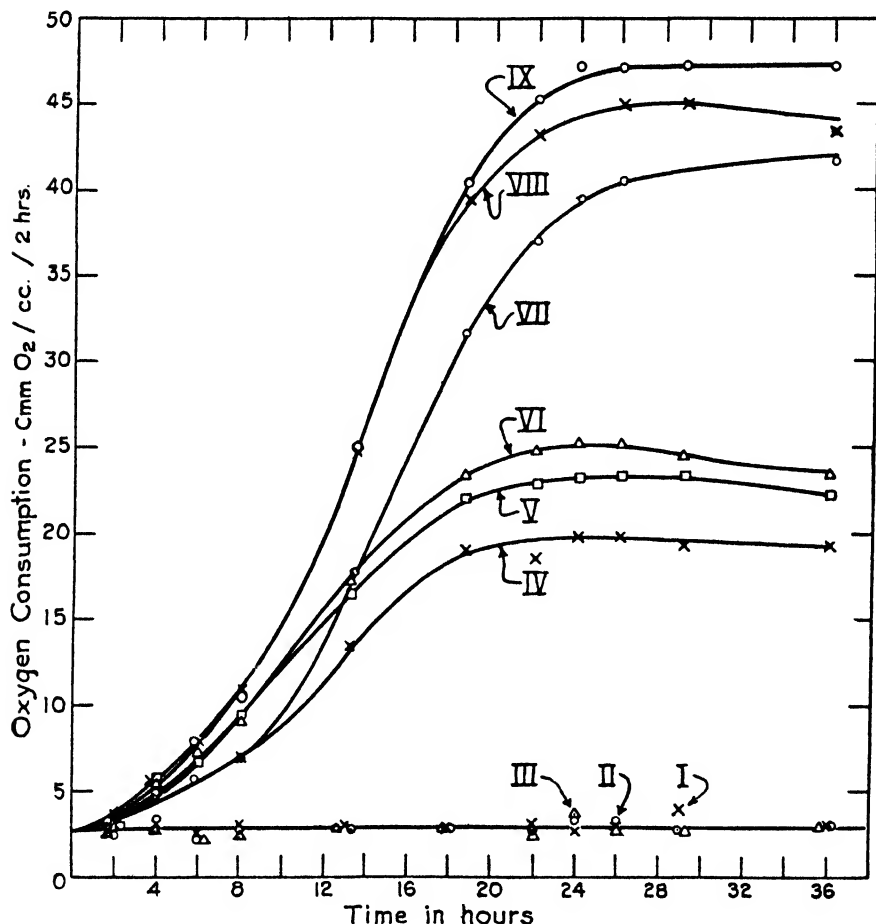


FIG. 1. THE EFFECT OF NATURAL AND SYNTHETIC HUMIC ACIDS AND OF COENZYME R UPON THE RESPIRATION OF *RH. MELILOTI*

I. Control. II. Synthetic humic acid (Fe = <0.01 per cent) 20 p.p.m. III. Synthetic humic acid (Fe = 10 per cent) 20 p.p.m. IV. Natural humic acid, 500 p.p.m. V. Coenzyme R, 2.4 p.p.m. VI. Coenzyme R, 2.4 p.p.m. + synthetic humic acid (Fe = 10 per cent) 20 p.p.m. VII. Natural humic acid, 1200 p.p.m. VIII. Coenzyme R 10 p.p.m. + synthetic humic acid (Fe = <0.01 per cent) 20 p.p.m. IX. Coenzyme R 10 p.p.m. + synthetic humic acid (Fe = 10 per cent) 20 p.p.m.

dilute alkali. C.P. ferric sulfate is added to supply the iron. The accessory factor for rhizobia was extracted from commercial sucrose with absolute alcohol. More concentrated preparations have since (8) been obtained from cultures of *A. vinelandii*, which synthesizes this factor.

The usual test organisms used were *Rhizobium trifolii*, strain 205, and *Rhizobium meliloti*, strain 131, obtained from I. L. Baldwin of the University of Wisconsin. The culture of *A. vinelandii* was obtained from J. G. Lipman, N. J. Experiment Station; *A. chroococcum* came from N. R. Smith of the Bureau of Plant Industry.

In these studies no attempt was made to eliminate the traces of iron from the basal medium. Only the effect of humic acid, when added to the usual type of synthetic medium, was determined. Even the better grades of dextrose and sucrose ordinarily (9) contain about 0.000009 to 0.0004 per cent iron as impurities, and this is not easily removed.

#### PHYSIOLOGICAL STUDIES

*Experiment 1.* The data, given in table 1, are typical of many results obtained showing the growth response of *Rhizobium* and *Azotobacter* to natural

TABLE 1

*The effect of natural humic acid upon the numbers of Rh. trifolii and A. vinelandii in a medium containing purified sugars*

HUMIC ACID (Fe = 0.45 PER CENT), P.P.M. DRY MATTER	MILLIONS OF BACTERIA PER CUBIC CENTIMETER			
	<i>Rh. trifolii</i> *		<i>A. vinelandii</i> †	
	2 days	4 days	2 days	4 days
0	4	4	34	74
1			52	96
5	10	30	70	120
10			86	174
25	50		98	212
50			138	260
100	190		140	212
200	400	1200		
600	560	1760		

\* Medium: inorganic salts, 1 per cent dextrose, and sodium nitrate.

† Medium: inorganic salts, 1 per cent sucrose, and no combined nitrogen.

humic acid. The marked difference between the magnitude of the response with the two types of organisms, and also the quantity of humic acid required to give a half maximum effect, are very striking.

No appreciable growth of the clover organism occurred in the absence of humic acid, but increasing additions up to the maximum used (600 p.p.m.) caused increased numbers almost in direct proportion to the quantity added. At the higher rates of application, even within the short incubation period of 2 to 4 days, the growth was extremely heavy—1760 millions per cubic centimeter in one case in contrast to 4 millions in the control.

The growth response with *A. vinelandii* was of a very different type. A fairly good growth occurred in the control and a four-fold increase above this where an optimum quantity of humic acid was added. A half-maximum

growth was obtained with 5 p.p.m., whereas 50 p.p.m. gave approximately a maximum effect. The same concentration of humic acid produced only a relatively small effect upon rhizobia when contrasted with the effect of the maximum application of 600 p.p.m. These data are in agreement with the results previously published (1) showing that *Rh. trifolii* requires a growth-producing substance which is present in natural humic acid. They also show that, although *Azotobacter* does not require the factor, it does manifest a marked growth response to humic acid, which, as recently shown (5, 6, 7, 9), is due in large part to its available iron content.

TABLE 2

*The effect of humic acid and other sources of iron upon the growth of Rh. trifolii in a medium\* containing commercial sucrose*

TREATMENT	QUANTITY ADDED, P.P.M. DRY MATTER	MILLIONS OF BACTERIA PER CUBIC CENTIMETER			
		2 days	3 days	5 days	8 days
Check.....	0	60	200	400	840
Natural humic acid (Fe = 0.45 per cent).....	10	156	320	780	1000
Natural humic acid (Fe = 0.45 per cent).....	100	320	620	940	1380
Natural humic acid (Fe = 0.45 per cent).....	1000	500	840	2000	2500
Synthetic humic acid (Fe = 3.5 per cent).....	10	98	300	620	1040
Synthetic humic acid (Fe = 3.5 per cent).....	100	126	400	580	1000
Synthetic humic acid (Fe = 3.5 per cent).....	1000	126	420	610	620
Synthetic humic acid (Fe = trace).....	10	74	202	500	620
Synthetic humic acid (Fe = trace).....	100	110	320	610	680
Synthetic humic acid (Fe = trace).....	1000	106	320	620	640
Ferric citrate.....	1	62	210	620	1040
Ferric citrate.....	10	94	320	610	840
Ferric citrate.....	50	82	300	580	860
Ferric sulfate.....	1	58	300	620	1020
Ferric sulfate.....	10	68	300	620	800
Ferric sulfate.....	50	90	320	620	820

\* Medium: inorganic salts, 1 per cent commercial sucrose, and potassium nitrate.

*Experiment 2.* Table 2 shows a typical set of data obtained when various iron sources were tested on *Rh. trifolii* using a commercial sucrose which contained a considerable amount of coenzyme R.

The growth in the control under these conditions was good, in contrast to practically no growth in the control in the preceding experiment. Additions of natural humic acid up to the maximum used, 1000 p.p.m., again produced very heavy growths. Synthetic humic acid, which contained only a small amount of coenzyme R but a very high percentage of available iron, gave a two-fold increase in growth over the check in a few cases, but usually less than this. The synthetic humic acid, containing only a trace of iron, gave still smaller growth increases. Likewise, ferric citrate and ferric sulfate gave slight growth increases but in no case were the effects comparable to those produced by natural humic acid.

*Experiment 3.* The results of a direct comparison of the response of two species of *Rhizobium* and two of *Azotobacter* to synthetic humic acid (Fe = 10 per cent) and to a preparation of coenzyme R, used singly and in combination with humic acid, are given in table 3. The synthetic humic acid, used alone, gave no increase in growth of *Rh. trifolii* and only a comparatively small effect with *Rh. meliloti*. The increased growth of *Azotobacter* was almost as large as that produced by natural humic acid (table 1). Coenzyme R, used singly, produced a good growth of the two species of *Rhizobium*, but the concentration used was insufficient for a maximum effect. *A. chroococcum* gave no response to the material, and *A. vinelandii*, only a slight increase. From other experiments, not reported here, it is evident that this slight stimulation was due to

TABLE 3

*The growth of Rhizobium and Azotobacter in the presence of synthetic humic acid and coenzyme R*

TREATMENT, P.P.M. DRY MATTER	MILLIONS OF BACTERIA PER CUBIC CENTIMETER						
	First experiment*			Second experiment†			
	<i>Rh. trifolii</i>	<i>Rh. meliloti</i>	<i>A. chroococcum</i>	<i>Rh. trifolii</i>	<i>Rh. meliloti</i>	<i>A. vinelandii</i>	
	5 days	5 days	5 days	4 days	4 days	2 days	4 days
Check.....	40	30	40	50	30	30	80
Humic acid 5.....				44	40	80	180
Humic acid 10.....	40	40	100				
Coenzyme R 10.....	120	200	40				
Coenzyme R 20.....				320	310	32	110
Humic acid 5† Co. R 20.....				400	400	62	160
Humic acid 10† Co. R 10.....	180	200	100				
Ferric Sulfate.....				40	36		
(Fe ≈ Fe in humic acid).....							

\* Basal Medium: inorganic salts, 1 per cent c.p. sucrose, and ammonium nitrate.

† Basal Medium: inorganic salts, 1 per cent c.p. sucrose (nearly Fe-free), and potassium nitrate.

a trace of iron present in the preparation. Where both the humic acid and growth substance were added the growth of the two strains of *Rhizobium* was on the average about 25 per cent greater than with coenzyme R alone present. This effect was presumably due chiefly to the available iron supplied by the synthetic humic acid. The growth of *Azotobacter* was approximately the same whether humic acid was used alone or together with coenzyme R. Ferric sulfate, where added to *Rhizobium* cultures containing no added accessory factor, produced no growth response.

*Experiment 4.* In an attempt to determine more accurately the importance of available iron for rhizobia the experiment reported in table 4 was carried out. A sample of natural humic acid and also one of a synthetic humic acid, prepared both with and without the addition of inorganic iron (9), were

tested separately and in the presence of varying amounts of the coenzyme preparation. The iron content of all flasks containing humic acid was 1 p.p.m., which is adequate to give a maximum effect with *Azotobacter*. The coenzyme preparation was not especially concentrated, hence the relatively high concentrations used.

Natural humic acid produced the usual marked increase in growth (10- to 20-fold), when compared with the control containing basal medium only. However, where the coenzyme preparation was present, the additional effect of the natural humic acid was relatively small (25-35 per cent increase). The sample of natural humic acid to which 9.5 per cent additional iron was added produced only a slight increase in growth; in fact, a growth response corresponding approximately to the natural humic acid added and not to the iron content.

TABLE 4

*The effect of natural and synthetic humic acids upon the growth of Rh. trifolii in media containing varying concentrations of coenzyme R*

HUMIC ACIDS ADDED, P.P.M. DRY MATTER	MILLIONS OF BACTERIA PER CUBIC CENTIMETER ON 3rd DAY Additions of Coenzyme R, p.p.m. dry matter			
	0	40	80	160
Check.....	42	300	500	700
Natural humic acid 200.....	460	560	680	860
Natural humic acid (with added iron) 10.....	66	320	500	780
Synthetic humic acid, low Fe, 100.....	24	280	480	720
Synthetic humic acid, high Fe, 10.....	22	300	500	600

Note: The iron content of all flasks containing humic acid was the same—1 p.p.m. The basal medium contained inorganic salts, 2 per cent c.p. sucrose, and potassium nitrate.

The data of table 4 furnish little evidence that *Rh. trifolii* needs highly available iron in appreciable amounts. The basal medium used, made from c.p. sucrose and c.p. salts and with no added iron, contained practically all of this element needed. The data do show very conclusively, however, that *Rh. trifolii* requires a growth factor. If the experiment had been continued for a longer time so as to obtain heavier growths the need for iron might have been more in evidence.

*Experiment 5.* The Warburg respiration technique, using a heavy suspension of the organisms, was used in a number of experiments. Figure 1 gives a typical set of results. The data are reported in terms of the volume of oxygen consumed per cubic centimeter of culture per 2-hour period. The increase in oxygen consumption with time, over this 36-hour interval, is a measure of growth.

In the basal medium, containing none of the necessary growth substance (curve I), the respiration remained essentially constant at the very low value corresponding to that of the heavy inoculum over the period of the experiment.

The addition to this medium of synthetic humic acid, whether free from iron (curve II) or whether containing iron (curve III), had no appreciable effect on growth or respiration in the absence of coenzyme R. In contrast, the addition of natural humic acid at the rate of 500 p.p.m. (curve IV) produced a marked increase in respiration which reached a maximum in about 20 hours. Likewise, the coenzyme preparation, used alone (curve V), caused a rapid increase in oxygen consumption. The curve in this case was similar to that of natural humic acid (curve IV) except that the concentration used happened to give a somewhat higher oxygen utilization. Where both the coenzyme preparation and high-iron synthetic humic acid were used together (curve VI) the total oxygen consumption was greater than with the concentrated growth substance alone. Although this additional growth, presumably due largely to the iron in the synthetic humic acid, was definitely positive, the increase was less than 10 per cent. At higher concentrations (curves VII, VIII, IX) the iron effect was approximately the same as at the lower concentrations (curves IV, V, VI).

Figure 1 shows, therefore, that it is the bacterial growth substance in natural humic acid which is responsible for most (at least 90 per cent) of the increased growth of *Rh. meliloti*, following its addition to the medium. The remaining effect is due to the other constituents present, chiefly available iron.

In previous publications the authors have pointed out that one of the unusual characteristics of coenzyme R is that its addition to a culture frequently causes a two- to four-fold increase in the rate of respiration during the first few hours before an increase in numbers occurs. In similar experiments natural humic acid gave a two- to three-fold effect and at the concentrations used was only slightly lower than the corresponding figures for the coenzyme preparation. This is further evidence that the important constituent in the two materials for rhizobia is the same.

It should be pointed out that a number of investigators (4) have shown that either molybdenum or vanadium is essential for nitrogen fixation by *Azotobacter*. Horner and Burk (9) and Burk (3) likewise observed that a portion of the effect of various iron sources on *Azotobacter* may, in some instances, be due to traces of molybdenum or vanadium present as impurities. Experiments in which we added these elements directly to the medium showed no growth response by rhizobia. The effect of other elements present as impurities in the iron sources used was not investigated.

#### SUMMARY

Experiments with two species of legume nodule bacteria, *Rh. trifolii* and *Rh. meliloti*, are reported, showing that the addition of natural humic acid to a synthetic (sugar-inorganic salts-nitrate) medium produces a marked increase in bacterial numbers and oxygen consumption. The growth obtained, over the concentration range of 0-600 p.p.m. dry matter, was nearly propor-

tional to the quantity added. In the absence of humic acid, and where a very pure sugar was used, no appreciable growth occurred.

Synthetic humic acids, prepared by boiling sugar in sulfuric acid, failed to produce a corresponding growth response on rhizobia. This held true regardless of the iron content of such preparations.

The growth response of the clover and alfalfa nodule bacteria to natural humic acid is due almost wholly to the presence of an essential growth substance, and not to the available iron content. This substance, coenzyme R, is essential for respiration and growth of these organisms.

The iron requirements of rhizobia are small. The stimulating effect of additions of available iron was usually less than 10 per cent in a synthetic medium prepared from C.P. ingredients.

*A. chroococcum* and *A. vinelandii*, used in comparative studies, showed no growth response to coenzyme R.

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# NUTRITION OF BLUEBERRY (*VACCINIUM CORYMBOSUM* L.) IN SAND CULTURES<sup>1</sup>

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The purpose of this investigation was the determination of some of the fundamental nutritional requirements of the blueberry plant (*Vaccinium corymbosum* L.) by laboratory and greenhouse experimentation. Such facts, it is felt, may be applied to conditions in the field to assist in arriving at an improved fertilizer practice for cultivated blueberries.

The fertilization of New Jersey blueberry soils has received attention since 1920 when Beckwith (1) reported favorably on a complete fertilizer. Revisions of this mixture have been recommended by Beckwith and Coville (3) and by Beckwith and Doehlert (4). The fundamentals of commercial blueberry culture were first determined by Coville (8), who reported in 1910 on extensive experiments showing this plant's peculiar need for an acid, peaty soil abundantly supplied with moisture but also well aerated. The application of these principles was pioneered by New Jersey growers who still produce almost the entire output in this new industry. The soil used is a native peat plowed and mixed with the sand subsoil. The locations selected for this purpose are practically all on St. John's sand or on swamp as classified by Lee, *et al.* (13). The reaction of these soils is reported as pH 3.5 to pH 4.5 by Beckwith (2) in a description of cranberry soils.

## METHODS

The sand culture studies with blueberries were started May 12, 1932, in the plant physiology greenhouses. The main series of 20 cultures was carried through three growing seasons including two fruit harvests, with the loss of one culture (number 10) due to spray injury. Blueberry cuttings (*Vaccinium corymbosum* L.) of the Rubel variety, which had been rooted one year previous in Holland peat, were used. The peat was carefully washed from the roots, and the cuttings were planted in pairs in 3-gallon percolator urns filled with washed quartz sand. The plants were watered daily with tap water for one week before any nutrient treatments were applied.

The technique of applying nutrients to these cultures is that described and used by Spencer and Shive (20) in their studies of the nutrition of another

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology and the cranberry and blueberry substation.



ericaceous plant, *Rhododendron ponticum* L. The same four salt solutions were used except that in the present study they were made up to an osmotic concentration of one-half atmosphere instead of one atmosphere as used by Spencer and Shive. The components of these solutions are shown in table 1. The solution numbers listed in column two of this table designate 20 representative solutions selected from the Tottingham (23) series of 84. These numbers refer to the position which the solutions and cultures occupy in the four-coördinate scheme employed by Tottingham to represent the series in diagram. This table also shows the partial volume-molecular concentrations

TABLE 1

*Partial volume-molecular concentrations of  $KH_2PO_4$ ,  $Ca(NO_3)_2$ ,  $MgSO_4$ , and  $(NH_4)_2SO_4$  in the solutions used\**

CULTURE NO.	SOLUTION NO.	GRAM MOLECULES PER LITER OF SOLUTION			
		$KH_2PO_4$	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
1	T <sub>1</sub> R <sub>1</sub> C <sub>1</sub>	.00105	.00073	.00829	.0007
2	C <sub>3</sub>	.00105	.00219	.00592	.0007
3	C <sub>5</sub>	.00105	.00365	.00355	.0007
4	C <sub>7</sub>	.00105	.00511	.00118	.0007
5	R <sub>3</sub> C <sub>1</sub>	.00105	.00073	.00592	.0021
6	C <sub>3</sub>	.00105	.00219	.00355	.0021
7	C <sub>5</sub>	.00105	.00365	.00118	.0021
8	R <sub>5</sub> C <sub>1</sub>	.00105	.00073	.00355	.0035
9	C <sub>3</sub>	.00105	.00219	.00118	.0035
10	R <sub>7</sub> C <sub>1</sub>	.00105	.00073	.00118	.0049
11	T <sub>3</sub> R <sub>1</sub> C <sub>1</sub>	.00316	.00073	.00592	.0007
12	C <sub>3</sub>	.00316	.00219	.00355	.0007
13	C <sub>5</sub>	.00316	.00365	.00118	.0007
14	R <sub>3</sub> C <sub>1</sub>	.00316	.00073	.00355	.0021
15	C <sub>3</sub>	.00316	.00219	.00118	.0021
16	R <sub>5</sub> C <sub>1</sub>	.00316	.00073	.00118	.0035
17	T <sub>5</sub> R <sub>1</sub> C <sub>1</sub>	.00527	.00073	.00355	.0007
18	C <sub>3</sub>	.00527	.00219	.00118	.0007
19	R <sub>3</sub> C <sub>1</sub>	.00527	.00073	.00118	.0021
20	T <sub>7</sub> R <sub>1</sub> C <sub>1</sub>	.00738	.00073	.00118	.0007

\* Osmotic concentration of each solution 0.5 atmosphere.

(gram-molecules per liter of solution) of the salts as they were used. In tables 2-4 the solutions are described by the brief method of referring to the proportional contribution of each salt to the total osmotic concentration.

Following the plan for rhododendron, the modification of Tottingham's series by Jones and Shive (12) was used in these experiments also. This requires the substitution of ammonium sulfate for potassium nitrate in equivalent partial osmotic concentration.

The solutions were applied to the surface of the sand by the continuous drip method set up as by Shive and Stahl (19). The rate of flow averaged 1,100

cc. a day. Iron was supplied in the form of ferrous sulfate in the proportion of 0.5 p.p.m. Tap water was used once a week for flushing in order to avoid undue accumulation of salts due to loss of water by transpiration and by evaporation from the surface of the sand and also to prevent undue changes of pH due to differential ion absorption. After the first seven weeks boron was supplied in the form of boric acid, and manganese, as manganese sulfate, both elements in the proportion of 0.5 p.p.m.

Four pairs of additional cultures were supplied with solution  $T_1R_1C_6$  at each of four different concentrations; viz., 0.25, 0.50, 0.75, and 1.00 atmosphere. After two seasons the cultures at the highest concentration had made somewhat less growth than those at the other three concentrations, but other differences were not markedly significant.

For two winter periods, September 26, 1932, to March 19, 1933, and September 2, 1933, to April 19, 1934, the plants were wintered in outdoor shelters just outside the greenhouses. For the growing season of 1933 the cultures were returned to the greenhouse and treated as before. There had been a light set of fruit buds. When the blooms opened they were hand pollinated at 2- or 3-day intervals from April 6 to 19, inclusive. A small crop of fruit was harvested in 1933. The data for this crop are not tabulated here since the larger yields of 1934 present more significant differences.

During the growing season of 1934, the plants were kept in an outdoor shelter, screened with tobacco cloth, at the cranberry and blueberry substation. Advantages prompting this move were additional facilities for daily care and observation and for cross pollination by bees. Some of the plants were 4 feet tall at this time with an equal spread laterally for the combined tops of the paired plants. But there was no evidence of any pot-bound condition either at this time or when the roots were removed from the urns at the end of the experiment. At this time the constant drip method of renewing the solutions was discontinued and the solutions were poured upon the surface of the sand in the morning and the late afternoon. Each culture was supplied with the same solution as before. Because of weather changes, the quantity varied from 1 to 2 liters a day, and during wet weather, applications were occasionally omitted. More than two consecutive applications were never omitted, however. When rainfall was not frequent the cultures were flushed with tap water.

It has already been noted that boron and manganese were not applied during the first seven weeks of experimental treatment. This lapse of time was required to reveal deficiency symptoms, which were characterized by the browning and killing of the tips of a few rapidly growing shoots and the appearance of irregularly shaped red-brown areas on some leaves. Within a few days after application of boron and manganese, growth was again normal, and no recurrence of these symptoms took place in the series of cultures 1-20.

Two available extra cultures not included in the main series or in the series of eight cultures used to test the four concentrations were used as checks, from

which boron and manganese were again withheld. The symptoms noted before reappeared. Addition of the two elements again resulted in normal growth. Once more in this same growing season this process was repeated. After wintering out of doors, these cultures were returned to the greenhouse and allowed to fruit. One continued to receive boron and manganese, while the other was again deprived of these elements. The former culture matured normal fruit, but the berries on the latter wilted slightly when half ripe and then slowly shriveled. Seed development in these shriveled berries was poor and meager. No cultures were set up which separated the effects of the two elements.

Deficiency symptoms in other plants caused by the lack of boron have been described by Warington (24), Brenchly and Warington (5), Johnston and Dore (10), McHargue and Calfee (16), and McMurtrey (17). Deficiency symptoms caused by the lack of manganese have been described by McHargue (14, 15). There is at present, in the literature, no reference to such deficiency studies with the blueberry.

From the findings of these workers, it would be reasonable to assume that the brown discoloration of leaves and the destruction of the growing tips of the blueberry were due to an insufficient supply of boron, whereas the failure of one culture to ripen fruit and mature seeds was, perhaps, evidence of the unsatisfied need for manganese. No symptom such as these have been noted in the blueberry plantations.

#### RESULTS

Many of the experimental plants made excellent growth and yielded good crops of fruit. None were stunted in growth, and all produced some fruit. Field plants bearing 370 grams of ripe berries to the bush in the third season after propagation, and accompanied by good vegetative growth, would be considered thrifty plants (cf. table 2).

The harvest of fruit from the experimental plants in 1934 was accomplished in ten pickings of ripe berries, distributed over a period of two months. These yields were recorded for individual plants as fresh weights, since facilities for drying so many lots of material were not available at the substation. As the ripe fruit was removed from each bush it was immediately weighed and recorded.

The average yields per culture are arranged in order of descending magnitude in table 2, which also shows, adjacent to each average yield, the osmotic proportions of the salts in the nutrient solution used. The five high-yielding cultures grouped at the top of the table are characterized, with respect to treatment, by low mono-potassium phosphate. With the exception of culture 1, they are also characterized by high nitrogen.

The dry weights of the tops, exclusive of fruit, are similarly arranged in order of descending magnitude in table 3. The five highest of these are also

characterized by high nitrogen and, except for culture 18, by low mono-potassium phosphate.

The blueberry naturally overbears. This results in a sharp reduction of leaf area, shoot growth, and food reserves in the current season and, further, in a smaller crop in the following season. Fruit production is the ultimate purpose of the grower. But the simultaneous production of foliage and new wood so largely determines the next crop that the two indexes of growth are

TABLE 2  
*Actual fresh weights and relative weight values of berries harvested*

CULTURE NO.	WEIGHT OF FRUIT HARVESTED			RELATIVE WEIGHT VALUES*	RELATIVE OSMOTIC PROPORTIONS OF THE SALTS IN THE SOLUTIONS USED			
	Plant I	Plant II	Average		KH <sub>2</sub> PO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
	gm.	gm.	gm.					
9	...	461	461	147	1	3	1	5
7	473	324	398	127	1	5	1	3
3	243	484	363	116	1	5	3	1
4	328	364	346	110	1	7	1	1
1	303	326	314	100	1	1	7	1
5	282	308	295	94	1	1	5	3
2	338	202	270	86	1	3	5	1
11	104	408	256	82	3	1	5	1
15	224	...	224	71	3	3	1	3
8	125	204	165	52	1	1	3	5
18	158	...	158	50	5	3	1	1
19	114	178	146	46	5	1	1	3
13	119	150	134	43	3	5	1	1
16	201	53	127	40	3	1	1	5
12	...	120	120	38	3	3	3	1
6	125	51	88	28	1	3	3	3
14	59	108	83	26	3	1	3	3
17	82	30	56	18	5	1	3	1
20	44	...	44	14	7	1	1	1

\* Computed on the basis of culture 1 as 100.

practically of equal importance in the evaluation of nutritional effect. Therefore, to obtain a truer picture of plant performance in response to nutrition, the relative production values for fruit and tops have been combined and averaged. It is not very likely that the equal weighting of these two values expresses exactly the ratio of their importance, but in the absence of quantitative data the method is adopted as the one most consistent with our experience with this plant. Relative values for yield of fruit and tops combined are given in table 4. It is particularly significant that here the best five cultures.

without exception, are those receiving treatments low in mono-potassium phosphate and high in nitrogen. Conversely, the solutions applied to the poorest yielding group of five cultures are characterized by high phosphate and low nitrogen. The high-yielding group is also characterized by treatments in which high proportions of calcium nitrate and, generally, low proportions of ammonium sulfate predominate.

TABLE 3  
*Actual dry weights and relative dry weight values of plant tops*

CULTURE NO.	DRY WEIGHT OF TOPS			RELATIVE WEIGHT VALUES*	RELATIVE OSMOTIC PROPORTIONS OF THE SALTS IN THE SOLUTIONS USED			
	Plant I	Plant II	Average		KH <sub>2</sub> PO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>					
3	227	309	268	191	1	5	3	1
18	252	...	252	180	5	3	1	1
7	302	194	248	177	1	5	1	3
6	283	203	243	174	1	3	3	3
2	231	227	229	164	1	3	5	1
4	184	263	224	160	1	7	1	1
9	...	222	222	158	1	3	1	5
13	177	213	195	139	3	5	1	1
11	137	203	170	122	3	1	5	1
15	168	...	168	120	3	3	1	3
8	131	184	158	113	1	1	3	5
5	140	156	148	106	1	1	5	3
20	143	..	143	102	7	1	1	1
16	201	81	141	101	3	1	1	5
1	149	131	140	100	1	1	7	1
19	122	106	114	82	5	1	1	3
14	58	170	114	81	3	1	3	3
12	...	108	108	77	3	3	3	1
17	105	75	90	65	5	1	3	1

\* Computed on the basis of culture 1 as 100.

#### DISCUSSION

There is an unusually close relation between the results obtained with these sand culture studies and the results of field work carried out by Beckwith and Doehlert (4). Field experiments conducted by these workers showed the greatest return of fruit with the use of nitrate of soda alone, but they conclude that the benefit of a complete fertilizer may have been obscured by excessive applications of rock phosphate. This is in agreement with the present sand culture experiments, which have shown maximum production by cultures receiving solutions low in mono-potassium phosphate and high in nitrogen. Further sand culture studies with greater replication are desirable.

Because of this agreement with field experiments and also because of the sandy nature of blueberry soils, it was felt that the methods of these experiments might be applicable to fertilizer investigations using ordinary blueberry soil. During the third season, therefore, a few plants potted in soil were treated regularly with solution  $T_1R_1C_7$  (table 1). The growth obtained was so encouraging that it seems very likely that studies of this sort with typical blueberry soil and commercial fertilizer salts could assist in solving the problem of economical and effective fertilization of blueberry plantings. Such studies

TABLE 4  
*Comparison of relative production values for tops and fruit combined*

CULTURE NO.	RELATIVE WEIGHT VALUES			RELATIVE OSMOTIC PROPORTIONS OF THE SALTS IN THE SOLUTIONS USED			
	Tops	Fruit	Average	$KH_2PO_4$	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
3	191	116	153	1	5	3	1
7	177	127	152	1	5	1	3
9	158	147	152	1	3	1	5
4	160	110	135	1	7	1	1
2	164	86	125	1	3	5	1
18	180	50	115	5	3	1	1
11	122	82	102	3	1	5	1
6	174	28	101	1	3	3	3
1	100	100	100	1	1	7	1
5	106	94	100	1	1	5	3
15	120	71	95	3	3	1	3
13	139	43	91	3	5	1	1
8	113	52	82	1	1	3	5
16	101	40	70	3	1	1	5
19	82	46	64	5	1	1	3
20	102	14	58	7	1	1	1
12	77	38	57	3	3	3	1
14	81	26	53	3	1	3	3
17	65	18	41	5	1	3	1

are now under way at the New Jersey Cranberry and Blueberry Substation. The continuance of the substation's field experiment which now includes a treatment very low in rock phosphate should be a helpful parallel.

In the middle group of nine cultures shown in table 4, there are four which received solutions low in phosphate. Of these four, two (cultures 1 and 5) received solutions low in nitrogen and high in magnesium sulfate, while the other two (cultures 6 and 8) received solutions fairly high in both nitrogen and magnesium sulfate. The evidence can be taken as a suggestion that the plant's need for magnesium is slight and that its growth processes may be easily retarded by an excess over this need.

The superiority of nitrate nitrogen over ammonium nitrogen is not surprising, inasmuch as the reaction of the nutrient solutions is about pH 4.5. The leachings are often as acid as pH 3.4. Various investigators, such as Tiedjens and Robbins (22), Tiedjens and Blake (21), Clark and Shive (7), and Davidson and Shive (9), have shown for several plants that anion nitrogen is absorbed more readily from a medium at relatively low pH than from a medium at relatively high pH, whereas cation nitrogen is absorbed more readily from a medium at high pH than from one at low pH.

The effect of potassium is not separated from that of phosphate. Shive (18) has conducted extensive investigations with soybeans using monobasic phosphates. In many cases injury occurred which he attributed not to the cations (Na,  $\text{NH}_4$ , K, Ca, and Mg) but to the common group  $\text{H}_2\text{PO}_4$ , or to the ions resulting from the partial dissociation of this group.

The blueberry is apparently more sensitive to variations in proportions of mono-potassium phosphate than is the rhododendron. Spencer (unpublished data) reports for rhododendron that "No injurious effects of solutions high in mono-potassium phosphate were evident. On many of the plants receiving high phosphate solutions the leaves presented a slightly wavy appearance along the margins instead of spreading out perfectly smooth and flat as in the case of the other leaves. This condition of the leaves, however, could hardly be regarded as detrimental to the plant since it was scarcely noticeable in most instances except only after very close inspection."

Other investigators at widely separated points working on different soils with blueberries in field plots have reported on the effect of phosphatic fertilizer. Johnston (11) in Michigan field tests with *Vaccinium corymbosum* did not find evidence of a low phosphorous requirement for blueberries. In his investigations, applications of superphosphate as heavy as 670 pounds to the acre resulted in profitable increases of yield, as compared with treatments with half as much superphosphate. On the other hand, Chandler and Mason (6) state that, in Maine, wild blueberries (*Vaccinium pennsylvanicum*) responded very favorably to fertilizers furnishing nitrogen, whereas "the plants on the plots receiving only phosphorous and potash were not much better than those on the untreated plots."

#### SUMMARY

Sand culture experiments with four-salt solutions were used to determine the nutritional needs of the cultivated blueberry (*Vaccinium corymbosum* L.).

Good vegetative growth and fruit production, as compared with field standards, were obtained.

The best nutrient solutions for the blueberry were low in phosphate and high in nitrogen.

Nitrate nitrogen under the conditions of these experiments was of greater value than ammonium nitrogen for this particular species.

There is agreement between the results obtained by the sand-culture,

nutrient-solution method of growing plants and the results obtained with blueberries in the field.

The need for boron and manganese was shown. Deficiency symptoms appeared during relatively short periods when these elements were withheld and were promptly eliminated when they were supplied.

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# THE ESTABLISHMENT OF MOISTURE EQUILIBRIUM IN SOIL

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For a period of 5 years, fortnightly determinations of moisture content have been made at the Desert Laboratory in an alluvial clay soil at eight depths to 6 feet, with biennial determinations to 12 feet. The results for the first 40 months have been published by the senior author.<sup>1</sup> The soil in question has been found to exhibit rather slow changes with reference to both increase and decrease of moisture content. At a depth of 6 feet the moisture content varies less than 2 per cent during periods of 1 to 2 years.

In connection with this work the question arose as to the length of time that would be required for two immediately contiguous bodies of soil with different initial amounts of water to come to equilibrium, resulting in uniform moisture content throughout the two bodies. The ideal conditions for the answering of this question would require that both bodies of soil be protected from any addition of water by rainfall and any loss by evaporation and also that they be protected from changes of temperature and barometric pressure. A simple experiment was devised in which the addition and loss of water were prevented but in which temperature and pressure were not controlled.

The plan of the experiment was to make up several lots of soil to known percentages of water content, to place two or three layers of widely different content in large cans, and to sample the cans at intervals to discover the extent to which the establishment of equilibrium was taking place.

The soil used was one which closely resembles a large percentage of the agricultural soils of Arizona—an alluvial clay, or adobe, from the flood plain of the Santa Cruz River, near Tucson. The mechanical composition of the soil is described in the paper just cited, its most important feature being that from 78 to 89 per cent will pass a 0.05-mm. sieve. The maximum water holding capacity of the lot of soil used in the experiment was 33.2 per cent, but other samples of the same type of soil run as high as 49 per cent. A quantity of the soil was air dried. (It then had 1.1 to 1.4 per cent of moisture as determined from oven-dried samples, or an average of 1.3 per cent.) Each sample was made up to known moisture content by thorough mixing, placed in the can a little at a time, and thoroughly tamped into place with a heavy iron 8 inches in diameter. The top of each layer was level, smooth, and firm before any

<sup>1</sup> Shreve, Forrest 1934 Rainfall, runoff, and soil moisture under desert conditions. *Ann. Assoc. Amer. Geogr.* 24: 131-156.

material for the next layer was placed on it. Stout garbage cans 60 cm. in depth by 35 cm. in diameter were used. Two cans were filled with two layers only and two others cans with three layers. The soil was brought to within 8 cm. of the top of the can and covered with a disk of galvanized iron resting on the soil. The disk was sealed to the wall of the can with plastilene, which was easily removed and replaced whenever the cans were opened for moisture determinations. The customary type of lid with deep flange was placed on each can. The cans were left outdoors in the complete shade of a small building throughout the experiment. The extreme annual range of air temperature to which they were subjected was, however, about 85°F. The sampling of the cans was done with a borer of 20 mm. diameter specially constructed for the purpose. In the cans with two layers, three samples were taken in each layer, and in those with three layers, two samples in each layer. The samples were 5 cm. in length and were so taken as to avoid the immediate vicinity of the line of junction between layers. The hole made by sampling was filled with soil closely approximating the moisture content of the soil removed, which could be estimated by color, and was thoroughly tamped into place. Each boring was made vertically from a different spot on the soil surface.

Since the initial water content of each can was known, it was possible to determine the error of sampling in each boring by averaging the six determinations made in each can. Most of the averages are close to the known moisture content, but some of them are high. It is evident that the error of sampling was not constant. A discrepancy of 1.3 per cent is to be expected because the layers were originally made up from air-dry soil whereas the moisture of the borings was determined from oven-dried material. It is probable that there was some horizontal movement of moisture, which might have been aided by the temperature changes to which the cans were subjected, and that some of the samples were not representative of the horizon from which they were taken. The duration of the experiment suggested the possibility that considerable moisture might have been lost from the surface layer by leakage and by evaporation at the time the cans were opened for sampling. After the close of the experiment two of the cans were opened and their entire moisture content determined by bringing the soil to air-dry condition. Can 4 was one of the two with driest surface layer. The borings taken in it at the end of the second year indicated an average moisture of 5.5 per cent, as compared with the initial 5 per cent. The actual moisture content for all of the soil in this can was 4.7 per cent (air-dry), indicating a small loss. Can 1 had the wettest surface layer. The borings in it at the end of the second year indicated an average of 15.2 per cent moisture, as compared with the initial 15 per cent. The entire body of soil was also removed from this can and found to have only 12.8 per cent moisture, indicating a much greater loss than in can 4. The failure of the final borings to indicate the actual loss by evaporation is probably due to the fact that no samples were taken in the uppermost 4 cm. of any cans at any of the readings.

In table 1 are shown the arrangement of the layers, their initial moisture contents, and the contents as determined on alternate months for the first year and at the end of the second year.

In can 1 there were two layers, the upper with 20 per cent, the lower with 10 per cent moisture. At the end of the first 2 months this can had gone half way

TABLE 1

*Percentages of initial and subsequent moisture content, on dry weight basis, for layers of soils, together with arrangement of layers and times of sampling*

CAN NUMBER	NOVEMBER, 1931	JANUARY, 1932	MARCH, 1932	MAY, 1932	JULY, 1932	SEPTEM- BER, 1932	NOVEMBER, 1932	NOVEMBER, 1933
1	20	17.6	16.1	15.5	16.0	17.0	19.2	16.3
		17.6	15.7	16.1	16.4	16.5	19.5	16.1
		17.4	17.0	16.0	16.6	17.2	19.5	16.5
	10	15.5	14.9	15.3	14.9	14.3	20.7	13.9
		16.0	14.9	14.1	14.3	14.8	16.7	14.4
		15.3	15.7	14.3	15.8	14.7	14.1	14.3
2	5	4.6	6.7	6.7	7.3	7.4	5.7	8.1
		5.3	7.9	8.3	9.6	8.7	7.1	8.6
		7.3	10.6	9.5	11.2	8.9	7.7	9.5
	15	13.0	13.8	13.5	13.8	13.6	12.2	13.1
		13.2	13.5	....	13.7	13.3	12.1	12.7
		13.2	13.5	13.2	13.4	....	....	....
3	15	13.1	13.5	13.0	13.1	13.1	11.2	12.2
		13.3	13.2	13.1	13.3	13.3	12.6	13.0
	10	11.3	12.6	11.3	11.9	11.7	10.1	12.2
		10.7	11.6	11.0	11.2	11.2	10.2	12.2
	5	7.6	8.9	9.3	9.4	9.8	7.6	11.8
		6.7	7.7	8.1	8.9	10.3	8.8	12.3
4	5	8.3	6.9	4.4	5.4	5.0	3.9	3.7
		6.2	5.1	4.7	5.1	4.5	4.2	3.7
	0	3.8	4.0	4.0	5.0	4.9	4.4	3.8
		4.5	5.3	5.3	6.7	6.9	5.1	4.5
	10	9.1	8.7	8.0	8.8	9.1	7.8	8.4
		10.7	9.3	7.4	8.6	9.1	7.9	8.7

toward the establishment of equilibrium; during the first year it showed little tendency to progress further toward equilibrium. Also there is little indication of the effect of gravity on the movement of moisture or of a localized change of moisture in either layer near the line of contact. The readings taken

in this can in November 1932 are evidently too high, whereas those taken in November 1933 are close to the known average and are very similar to those taken in July 1932. In short, during the 22 months between the first and last readings on can 1 it had made no greater progress toward the establishment of equilibrium than it did in the first 2 months.

In can 2 there were two layers, the upper with 5 per cent and the lower with 15 per cent moisture. At the end of the first period this can had not gone so far toward equilibrium as can 1, doubtless because the layer with higher initial moisture was below, instead of above, the drier one. All readings in can 2 show a higher moisture content in the bottom of the upper layer, where it was contiguous to the layer with 10 per cent greater moisture, but they fail to show a progressive tendency for the moisture from the 15 per cent layer to move toward the top of the 5 per cent layer. In a soil of such fine texture this behavior is probably due in only a slight degree to the influence of gravity, and is more probably the result of the absence of evaporation from the surface of the 5 per cent layer. There is evidence in the later readings that the upper layer became more uniform in its content, but there is little evidence of a continued loss from the 15 per cent layer to the 5 per cent layer. At the end of two years can 2 had not gone further toward equilibrium than can 1 had in the first 2 months, a difference due to the smaller moisture content in can 2, and possibly to the operation of gravity in can 1.

In can 3 there were three layers with 15 per cent, 10 per cent, and 5 per cent moisture respectively from top to bottom. In this can there was an opportunity for gravity to operate toward the establishment of equilibrium, which could be brought about only by the movement of moisture from the top layer to the bottom one through the middle layer, which was already at the average percentage for the can. In the first 2 months there was a movement which extended less than half way toward equilibrium. In the succeeding periods there was only a slight additional movement, involving an increase in the moisture of the 5 per cent layer at the expense of the central 10 per cent layer, with little effect on the upper 15 per cent layer. At the end of the first year the average of the six determinations was 10.1 per cent. The middle layer had returned approximately to its original content, and the top and bottom layers had gone a little more than half way toward equilibrium. The readings taken at the end of the second year are high, with an average of 12.3 per cent instead of 10 per cent, but they indicate the closest approach to moisture equilibrium shown in any member of the series, all six readings being much more nearly identical than in the determinations at the end of the first year.

In can 4 there were three layers with 5 per cent, 0, and 10 per cent moisture respectively from top to bottom. This can was planned to test the movements required to bring about equilibrium by the passage of water through a layer initially air dry. The readings taken at the end of the first 2 months showed an average of 7.1 per cent, which is much higher than the known average of 5 per cent, and the error appears to be chiefly in the readings for the top layer.

Throughout the history of this can there was a relatively small loss of moisture by the bottom layer, with some indications of a return of moisture to it from the middle layer. The middle layer fluctuated slightly but returned at the end of the second year to precisely the same readings obtained at the end of the second month. After May 1932 there was little change in the moisture in the top layer until November 1932. At the end of the second year there was an approximate equilibrium between the two upper layers, but the bottom layer showed no progressive change during the last 18 months of its history.

#### SUMMARY

Bodies of soil of known moisture content were firmly tamped into large garbage cans in two or three layers in each can, with differences of 5 per cent or 10 per cent between the contiguous layers. The soil was sampled on alternate months for 1 year and again at the end of the second year. The soil used was an alluvial clay, or "adobe," with a water-holding capacity of 33.2 per cent.

In one of the four cans there was an approximation to equilibrium at the end of the second year, the extreme range of the readings being 11.8 to 13.0 per cent. In the other three cans a uniform distribution of moisture through the two or three layers was not attained.

The change in distribution of the moisture during the first 2 months was greater than the total of all change in the subsequent 22 months in all cases but one.

In some of the changes there is evidence of the influence of gravity. The individual layers of soil in a few cases showed the influence of an adjacent layer in the samples taken nearest it and a weaker influence in the sample or samples taken farther from it.

The evidence of these results, and of those obtained in previous work on the natural moisture changes of soil in place, indicates that the movement at the low percentages prevailing in desert soils is very small, at least in heavy soils. The only rapid changes in moisture content are the increases due to heavy rainfall and the decreases at the surface by evaporation or at lower levels by root absorption. When low moisture contents are concerned and small differences of content are involved, the action of capillarity in the slow distribution of water through the soil and in the establishment of moisture equilibrium is of little importance.



# SOIL SAMPLING TUBES FOR SHALLOW DEPTHS

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During the course of a study of forest soils in the Southeast, made by the Southern Forest Experiment Station, it has been necessary to collect several thousand samples from the upper portion of the soil profile. Three distinct types of soil tubes (fig. 1) were designed for the specialized sampling used in this study. These are as follows:

- A: A tube for collecting samples for moisture determination of the 0 to 7-inch soil depth.
- B: A tube for collecting a large number of cores in undisturbed field condition from the 0 to 6-inch soil depth of a size large enough to grow tree seedlings.
- C: A tube for obtaining a large number of samples of constant volume from the 0 to 3-inch soil depth.

Since at least 200 samples have been collected with each tube, it has been possible to modify the original designs and thus correct imperfections. The tubes, as here described, have been found to be sturdy and easy to manipulate. They have been satisfactorily used to sample forest soils free from stones and varying in texture from fine sand to silt loam. For use in different regions or for sampling different depths it may be desirable to modify the general dimensions of the tubes as shown here.

## TUBE A

Tube A is used for obtaining a core of soil in relatively undisturbed condition. After the core is collected, one half of the tube may be removed in such a manner that the soil core remains in the other half in a convenient position for removal in its entirety or in portions. The tube enables collection of a core approximately 2 inches in diameter and 7 inches long.

*Operation.* The tube is driven into the soil with a wooden mallet until the lower rim of the top touches the soil surface. The cutting edge is then  $7\frac{1}{2}$  inches beneath the surface. A hole is dug on one side to a depth of 6 to 7 inches with either a trowel or small spade, and the soil core is broken by tilting the top of the tube sharply toward the hole. The tube is removed from the soil, and the top is removed by disengaging the locking pins with a slight twist of the top. The tube is then held horizontally in one hand and the upper half removed with the other, which exposes the sample so that it can be removed as desired. Small grooves may be cut along the edges of the bottom half of the tube to designate particular depths beneath the surface, thus eliminating the use of a ruler in measuring depths of the desired portions of each core.





This permits very accurate sampling with respect to depth. Very dry soils cannot be sampled with this tube.

The effective sampling depth of the tube here described is 7 inches. An extra half-inch is allowed for loss when breaking off the soil core. If the tube is driven into the soil past the lower edge of the top, soil particles may work between the top and the tube proper and make removal of the top difficult. Therefore, it is best to consider the effective sampling depth of the tube from the cutting edge to the lower edge of the top. The top should fit tightly, as otherwise there may be movement of the separate halves of the tube.

*Construction.* The tube described here was made from standard 2-inch steel pipe. In order to have a cutting edge of smaller diameter than the rest of the tube, to prevent compaction of the soil core and to provide a slight enlargement on the outside to facilitate driving the tube into the soil, both interior and exterior of the lower portion of the tube were built up with high test welding steel. The tube was then mounted on a lathe and turned to required dimensions. The heavy  $\frac{5}{16}$ -inch upper portion of the tube was similarly constructed. The total cost of the tube, including hardening, was \$8.

The upper portion of the tube must be considerably thicker than the  $\frac{1}{8}$ -inch wall of the lower portion to present a sufficiently strong bearing surface for the top, otherwise the rim will flatten out from constant pounding, and removal of the top will be difficult. The top is held firmly in place by means of two  $\frac{3}{16}$ -inch pins located on opposite sides of the upper portion of the tube. These engage with slots in the top. The slots should be large enough so that the top rests on the rim of the tube and not on the pins, which have little structural strength. The cutting edge should be case-hardened. It is also desirable, although not essential, to case-harden the upper edge which supports the top. The lock lugs are cut from  $\frac{1}{16}$ -inch steel and should be carefully welded on; these prevent the lower portions of the tube from separating. After being welded, they should be ground and smoothed on an emery wheel so that there are no projections on which roots or stones might catch and thereby bend or break the lugs.

#### TUBE B

This tube<sup>1</sup> was designed to provide a soil sample for seedling growth studies. It was necessary to collect the sample in a field condition as undisturbed as possible. The adaptibility of the tube for additional studies, such as volume weight determination, percolation rate, and others requiring undisturbed soil, is obvious. The tube was designed to accommodate a 1-quart pasteboard carton, with cover removed, placed into the tube mouth down.

*Operation.* After the carton is inserted, the top of the tube is locked in place by twisting slightly to engage the two lock pins in their slots on opposite sides of the top. The tube is then driven into the soil to the desired depth with a wooden mallet. After the tube is dug from the soil with a trowel, the carton

<sup>1</sup> The writer designed this tube from suggestions received from T. S. Coile, who had made use of a similar one. Although several such tubes apparently have been used, no detailed description of them appears to have been published.

is removed and covered, the sample then being in an inverted position inside the carton. If samples thus collected are to be kept in the container for several months, as in a growth study,<sup>2</sup> or if they are to be used in soil moisture studies, it is necessary to paraffin the interior of the cartons to avoid deterioration of the carton or loss of moisture from the soil sample. Moisture content of soil does not influence sampling with this tube.

*Construction.* The cutting edge of the tube must be case-hardened. Unlike tube A, it is not necessary to harden or enlarge the bearing edge of the tube which supports the top, since the rim around the bottom of the pasteboard carton projects above the edge of the tube for a fraction of an inch and breaks the force of the blow on the tube. The pasteboard rim is slightly flattened, but this neither disturbs the soil sample nor seriously weakens the carton. Since both tube and top are driven into the soil, a somewhat different design was used from that of tube A, as may be seen in figure 1. The tube and top were turned from solid blocks of cold rolled steel. The approximate cost, including hardening, was \$10.

Should it be desirable to use either a  $\frac{1}{2}$ -pint or a 1-pint pasteboard carton in the tube, this may readily be accomplished by turning out on a lathe a wooden cylinder of proper diameter to slip easily into the tube and of proper length to fill the space between the bottom of the carton and top of the tube. The sample is then obtained as already described for the larger sized carton.

#### TUBE C

Tube C was designed to provide a rapid means of collecting samples of constant volume. Samples collected with the tube here represented, 4 inches in diameter and 3 inches in height, afforded a convenient means of volume weight determination, since, as a constant volume of soil was always obtained, only the oven-dry weight was needed. The sampler consists of a tube and a plunger for forcing out the soil.

*Operation.* The tube is driven into the soil until the plunger rests against the top. The two  $\frac{5}{16}$ -inch holes, on opposite sides of the top, afford a ready means of observing the position of the plunger. The tube is then dug from the soil with a trowel and the superfluous soil carefully cut off with a long-bladed knife so that the soil surface coincides with the level of the cutting edge. The soil is removed from the tube by inserting a metal rod (a 60-penny spike with the point sawed off is excellent) into the hollow handle and driving it against the stem of the plunger, thus forcing the soil out of the tube. Prior to the collection of each sample, the tube should be well shaken so that all soil particles which pass by the edges of the plunger are removed. This is an important precaution when sampling moist soils. Moisture content of the soil being sampled does not affect operation of this tube.

*Construction.* This tube may be turned out of ordinary steel pipe. The cutting edge should be case-hardened. The total cost, including hardening, was \$4.

<sup>2</sup> In growth studies the upper side of the carton (actually the bottom) is cut off; the lid then becomes the bottom.

# A SIMPLE METHOD OF FINDING THE LIME STATUS AND LIME REQUIREMENT OF SOILS, BASED ON REACTION WITH $\text{CaCO}_3$

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Lime status (L.S.) of a soil may be defined as the ratio expressing as percentage the exchangeable Ca actually present in the soil ( $\text{Ca}_1$ ) over the exchangeable Ca the soil will have when brought into equilibrium with  $\text{CaCO}_3$  ( $\text{Ca}_2$ ), i.e.,

$$\text{L.S.} = \frac{100 \text{ Ca}_1}{\text{Ca}_2}$$

It might be stated at the outset that equilibrium with  $\text{CaCO}_3$  does not represent the saturation of the acidoid with Ca ions. Lime status, therefore, should not be confused with the state of saturation of the soil. The advantage of using the  $\text{CaCO}_3$  equilibrium point lies purely in its practical utility, for in nature no soil is more saturated with Ca than that point. The equilibrium point with  $\text{CaCO}_3$  has already been suggested as the basis for the lime requirement of soils (1, 2, 3, 5). On account of the limited solubility of  $\text{CaCO}_3$  its reaction with soils has an attractive simplicity, for a uniform condition can be easily maintained for all soils and no delicate adjustment is required for attaining the approximate equilibrium point.

The chief obstacle in the way of adopting the  $\text{CaCO}_3$  equilibrium point as the basis for defining lime status has been the difficulty of estimating exchangeable Ca in the presence of excess  $\text{CaCO}_3$ . Since the single treatment methods of determining exchangeable Ca, developed by the senior author (4), have rendered such estimations extremely simple, it was of interest to explore the possibilities of using the newer methods for finding the lime status of soils.

## EXPERIMENTAL

The starting point of this investigation was the potassium oxalate method of estimating exchangeable calcium in calcareous and carbonate-free soils (5). This method was slightly modified to suit the present conditions under which the soil suspension had to be shaken with  $\text{CaCO}_3$ . This consisted merely in using potassium oxalate-acetate-carbonate mixture of double the strength, every other detail remaining the same.

Some preliminary experiments were performed to study the conditions

under which equilibrium between  $\text{CaCO}_3$  and soils could be attained. These will be described first.

TABLE 1  
*Speed of reaction between  $\text{CaCO}_3$  and soil*

TREATMENT	TIME OF SHAKING	EXCHANGEABLE Ca
	hours	m.e.*
50 cc. $\text{H}_2\text{O}$ .....	2	6.0
50 cc. $\text{H}_2\text{O} + \text{CaCO}_3$ .....	2	11.0
50 cc. $\text{H}_2\text{O} + \text{CaCO}_3$ .....	24	11.3
50 cc. 0.2 N KCl + $\text{CaCO}_3$ .....	1	11.0
50 cc. 0.2 N KCl + $\text{CaCO}_3$ .....	1.5	11.2
50 cc. 0.2 N KCl + $\text{CaCO}_3$ .....	2	11.8
50 cc. 0.2 N KCl + $\text{CaCO}_3$ .....	2.5	12.8
50 cc. 0.2 N KCl + $\text{CaCO}_3$ .....	3	12.9
50 cc. 0.2 N KCl + $\text{CaCO}_3$ .....	24	12.2
50 cc. 0.2 N KAc + $\text{CaCO}_3$ .....	2	12.2
50 cc. 0.2 N KAc + $\text{CaCO}_3$ .....	24	12.6

\* Per 100 gm. soil.

TABLE 2  
*Speed of reaction between  $\text{CaCO}_3$  and soil under partial vacuum*

TREATMENT	TIME OF SHAKING	EXCHANGEABLE Ca
		m.e.*
50 cc. 0.2 N KCl (10 gm. soil).....	10 minutes	5.9
50 cc. 0.2 N KCl (15 gm. soil).....	10 minutes	5.9
50 cc. 0.2 N KCl (20 gm. soil).....	10 minutes	5.4
50 cc. 0.2 N KAc.....	10 minutes	5.5
50 $\text{H}_2\text{O} + \text{CaCO}_3$ .....	10 minutes	9.3
50 cc. 0.2 N KCl + $\text{CaCO}_3$ (10 gm. soil).....	10 minutes	9.9
50 cc. 0.2 N KCl + $\text{CaCO}_3$ (15 gm. soil).....	10 minutes	10.2
50 cc. 0.2 N KCl + $\text{CaCO}_3$ (20 gm. soil).....	10 minutes	9.2
50 cc. 0.2 N KCl + $\text{CaCO}_3$ (10 gm. soil).....	1 hour	11.8
50 cc. 0.2 N KCl + $\text{CaCO}_3$ (10 gm. soil).....	2 hours	9.4
50 cc. 0.2 N KCl + $\text{CaCO}_3$ (10 gm. soil).....	3 hours	9.8
50 cc. 0.2 N KAc + $\text{CaCO}_3$ .....	10 minutes	10.1
50 cc. 0.2 N KAc + $\text{CaCO}_3$ .....	1 hour	9.8
50 cc. 0.2 N KAc + $\text{CaCO}_3$ .....	2 hours	9.8
50 cc. 0.2 N KAc + $\text{CaCO}_3$ .....	3 hours	10.0

\* Per 100 gm. soil.

*Speed of reaction between  $\text{CaCO}_3$  and soil*

A laterite soil from Dacca (Bengal) was chosen for these preliminary experiments. About 1 gm. of  $\text{CaCO}_3$  was allowed to come to equilibrium with soil in the presence of water, potassium chloride, or, potassium acetate solution. Shaking was done occasionally with the hand for varying lengths of time.

After treatment with  $\text{CaCO}_3$ , the suspension was shaken with potassium oxalate-acetate-carbonate mixture, as described under "detailed description of the method," and exchangeable Ca was determined.

Another set of experiments was carried out in which the reaction between  $\text{CaCO}_3$  and soil was allowed to take place under reduced pressure to facilitate the escape of  $\text{CO}_2$ . The results of the two sets of experiments are given in tables 1 and 2, from which the following conclusions may be drawn:

Equilibrium between  $\text{CaCO}_3$  and soil suspension is attained fairly rapidly. One hour's shaking by hand at ordinary pressure and 10 minutes' shaking at reduced pressure are sufficient for the purpose.

The equilibrium point is not materially affected by allowing the reaction to take place in a solution of potassium chloride or potassium acetate instead of water.

The results are independent of the amount of soil taken; and 10 to 20 gm. of soil may be used for each determination.

The results in table 2 refer to a slightly different sample of the same soil, and the values are slightly lower than those in table 1. The difference, however, is well within the sampling errors which may normally be expected.

#### *Detailed description of the method of finding lime status*

Ten grams of soil is left with 50 cc. of water with and without  $\text{CaCO}_3$  (about 1 gm.). The suspension is occasionally shaken by hand for 2 or 3 hours or may be left overnight. During this shaking the stopper is kept loose to allow the  $\text{CO}_2$  to escape from samples to which  $\text{CaCO}_3$  has been added. The reagent bottles can also be connected to an ordinary water pump and the reaction allowed to take place under reduced pressure when equilibrium can be attained in about half an hour.

When the reaction is complete the suspension is cooled to  $10^\circ\text{C}$ . and 50 cc. of the following solution is added: 0.2  $N$  with respect to K oxalate,  $N$  with respect to K acetate, 0.03  $N$  with respect to K carbonate.

The suspension is shaken by hand for about half an hour while the temperature is kept round about  $10^\circ\text{C}$ . It is then filtered through a dry, fluted paper, and 50 cc. of the filtrate is titrated with standard permanganate. The total decrease in the concentration of oxalate ion is equivalent to the exchangeable Ca, and the lime status (L.S.) is calculated as follows:

$$\text{L.S.} = \frac{100 \text{ Ca}_1}{\text{Ca}_2}$$

where  $\text{Ca}_1$  = exchangeable Ca in the original soil, and  $\text{Ca}_2$  = exchangeable Ca in the soil after being shaken with  $\text{CaCO}_3$ .

#### *Lime status and lime requirement of soils*

The lime requirement of a soil may be taken as equivalent to  $(\text{Ca}_2 - \text{Ca}_1)$ , i.e., the amount of Ca taken up by the soil from  $\text{CaCO}_3$ . We can express this value in tons per acre as follows:

One acre, six inches weighs 2,000,000 pounds, which is equal to 1000 tons (one short ton being equal to 2000 pounds). One milliequivalent of CaO is equal to 0.028 gm. Therefore, an increase of 1 m.e. of exchangeable Ca per 100 gm. of soil on shaking with  $\text{CaCO}_3$  is equal to 0.28 tons of CaO per acre 6 inches. Lime requirement of a soil in tons per acre is equal, therefore, to  $(\text{Ca}_2 - \text{Ca}_1) \times 0.28$ . Of course this value has to be multiplied with a suitable factor according to the percentage of CaO in the given sample of lime; for instance, if liming is done with  $\text{CaCO}_3$ , lime requirement is equal to  $(\text{Ca}_2 - \text{Ca}_1) \times 0.5$ . It is interesting to note that the reaction between  $\text{CaCO}_3$  and soil is very rapid; hence, liming with  $\text{CaCO}_3$  should be as effective as with  $\text{Ca}(\text{OH})_2$ . In table 3, lime status and lime requirements of some typical Indian soils are given. No claim is made as to the application of these values to field conditions except in the case of soil 6, which was taken from a field the lime require-

TABLE 3  
*Lime status and lime requirement of soils*

LAB. NO., P. C.	LOCALITY	pH	Ca <sub>1</sub>	Ca <sub>2</sub> I	Ca <sub>2</sub> II	Ca <sub>2</sub> III	L.S. per cent	L.R. tons*
3	Dharwar (Bombay)	7.64	51.2	52.0	52.4	55.6	98.5	0.224
6	Dacca (Bengal)	5.29	3.8	9.6	10.0	9.2	39.6	1.62
9	Malabar (Madras)	5.76	1.4	8.6	....	6.0	16.3	2.02
12	Tacklai (Assam)	5.84	2.8	7.6	8.2	6.8	36.8	1.34
14	Estate soil (Madras)	5.37	6.8	17.0	18.6	15.0	38.6	2.86
15	Shillong (Assam)	7.71	11.6	15.6	15.0	14.4	74.3	1.12
20	Lower Burma	5.64	1.9	5.5	4.9	2.9	34.5	1.01
25	Bhin soil (U. P.)	7.41	0.8	3.1	3.6	2.8	25.8	0.64
34	Gurdaspur (Punjab)	7.63	4.6	4.2	6.4	4.6	100	0
40	Umareth (Bombay)	7.65	7.6	9.6	10.4	8.8	79.2	0.56
45	Bihar	7.47	2.8	3.9	5.0	3.6	71.8	0.31
46	Baroda (Bombay)	7.63	38.2	43.2	42.2	....	88.4	1.40
49	Madhopur (Bihar)	6.33	10.8	13.6	15.6	13.6	79.4	0.78

Ca<sub>1</sub> = Exchangeable Ca in original soil (m.e. per 100 gm.).

Ca<sub>2</sub>I = After treatment with  $\text{CaCO}_3$  in water solution for 24 hours.

Ca<sub>2</sub>II = After treatment with  $\text{CaCO}_3$  in KCl solution for 24 hours.

Ca<sub>2</sub>III = Ten minutes' shaking with water under reduced pressure.

L.S. and L.R. values on Ca<sub>2</sub>I basis.

\* Per acre 6 inches.

ment of which was actually determined by the author by growing a crop on it and was found equal to 1.3 tons per acre. The value of 1.62 tons per acre found by this method is sufficiently close to justify field trials with other soils.

It is important that the distinction between lime status and lime requirement be clearly borne in mind. Lime status is a ratio and is somewhat similar to state of saturation (but should on no account be confused with it) of soil and as such can be used for comparing soils from different localities with regard to their fundamental properties. Lime requirement, on the other hand, is a quantity that shows the deficiency of Ca in a particular soil and is not fundamentally related to lime status. In other words, two soils having the same lime status may have entirely different values for lime requirement. When

the lime status of a soil is less than 100 per cent we can say that it may require lime, but just how much per acre, we cannot say. It is, therefore, advantageous when recording the values of lime status of soils to give the values for  $\text{Ca}_1$  and  $\text{Ca}_2$  in milliequivalents per 100 gm. of soil. This will enable anyone to calculate lime requirement values if required.

The proposed method of finding the lime requirement of soils has the merits of simplicity and ease of manipulation. It is based on a sound principle, and therefore values obtained are not dependent on the amount of soil, the time of shaking, or the strengths of solutions. The precautions to be observed are no more than those proposed for the analytical procedure for the estimation of exchangeable calcium.

#### SUMMARY

A method of determining the lime status and lime requirement of soils is outlined. It consists in the estimation of exchangeable calcium before and after treatment with  $\text{CaCO}_3$ , by the potassium oxalate method.

Within limits, the results obtained are independent of the amount of soil, the strength of solution, or the time of shaking.

Results with some typical Indian soils are given by way of illustration.

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# THE RELATION OF CRYSTAL STRUCTURE TO BASE EXCHANGE AND ITS BEARING ON BASE EXCHANGE IN SOILS

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The colloidal fraction of the soil contains a high percentage of the total exchangeable bases of the whole soil, but a considerable part of the Mg of the colloidal fraction is not replaceable in the ordinary sense of the term, and the same is true in some cases, at least, of Ca, K, and Na. However, the discovery by Hendricks and Fry (3) and by Kelley, Dore, and Brown (9) that soil colloids are composed, in considerable part at least, of crystalline material, and that the base-exchange capacity of soil colloids can be increased merely by reducing the natural size of the individual particles that compose the colloidal fraction (9), raises this question: Is there any fundamental difference between the exchangeable and the non-exchangeable bases of such materials? It is possible that the only essential difference between these two parts of the base-containing-constituents of soil colloids is merely a matter of particle size. If this is true it would seem to indicate that exchangeability of the bases depends on whether the ions happen to be located on the exposed surface of the colloidal particles and not necessarily, either that different kinds of compounds occur in the colloidal particles or that the bases are held to the particles by different kinds of attractive forces.

Kelley, Dore, and Brown reported that, corresponding to the increase in base-exchange capacity produced by grinding, there is a parallel increase in the replaceable Mg and K and also H ions in certain cases. They found this to be true both of soil colloids and of certain clay minerals.

The discoveries just alluded to tend to focus attention on crystal structure as such and on the base-exchange properties of pure minerals. They also raise three rather broad general questions: First, of what specific minerals are the soil colloids composed? Second, what determines the base-exchange capacity of the different classes of minerals? Third, are the exchangeable cations of minerals present as essential lattice constituents, and if so, what relation do they bear to the other components of the lattice?

It has long been known that the natural zeolites have pronounced base-exchange properties. In addition Lemberg (11) showed a half century ago that analcite can be converted into leucite, and *vice versa*, through base exchange;

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Sullivan (17) and others have shown that a wide variety of the primary minerals undergo base exchange to a limited extent upon treatment with salt solutions. Despite these and other investigations, it is generally held that both the constituents of soils and pure minerals may be subdivided into two rather sharply defined classes; namely, those whose bases are replaceable and those whose bases are nonreplaceable.

Recently we have found that the base-exchange capacities of several minerals, some of which belong to one of the aforementioned classes and some to the other, tend to approach each other upon grinding the samples. For example, the exchange capacity of both orthoclase and muscovite can be raised to a comparatively high level (from 76 to 91.5 or more m.e. per 100 gm.) merely by grinding the sample to extreme fineness. What is still more surprising, the same is true of kaolinite and pyrophyllite. On the other hand, substances like quartz, colloidal  $\text{SiO}_2$ , precipitated  $\text{Al}(\text{OH})_3$ ,  $\text{Fe}(\text{OH})_3$ , bauxite, and talc, have only low base-exchange power, as determined by the  $\text{NH}_4$ -acetate method, however long they may be ground.

According to modern concepts of crystal structure [Bragg, (1) Goldschmidt, (2) and Pauling, (14)], each Si ion in the silicates is surrounded by four closely packed O ions arranged in the form of a tetrahedron. The relative sizes of O and Si ions ( $\text{Si} = 0.8 \text{ \AA}$ ,  $\text{O} = 2.7 \text{ \AA}$ ) make possible a very close packing of four O ions around each Si ion. In quartz each O ion is a part of adjacent tetrahedra, which are so arranged that the negative charges of the O ions are just balanced by the positive charges of the Si ions. Consequently, quartz is electrically neutral, except on the surface, and contains no bases in the ordinary sense. In other silicates, such as the amphiboles, some of the O ions lie between two Si ions and some are attached to Si ions on one side only. Such minerals always contain enough metal ions to balance the remaining charges of the O ions. In certain of the alumino-silicates, such as the feldspars and zolites, the Al ions ( $1.1 \text{ \AA}$ ) are also present in tetrahedra, being surrounded by four closely packed O ions. Since the Al ion bears only three positive charges, these minerals must contain additional cations (bases), sufficient to balance the excess negative charge of the Al tetrahedra. Potassium performs this function in orthoclase, biotite, and muscovite; sodium plays a similar rôle in albite, analcite, and natrolite; and calcium in anorthite and scolecite.

On the other hand, according to Pauling (15), each Al ion of pyrophyllite is surrounded by four O ions and two OH ions; in kaolinite each Al ion is surrounded by two O ions and four OH ions; that is, Al in these minerals has a coördination number<sup>3</sup> of 6. In these minerals the Al is said to occur in the octahedral position, and the positive charges of the Si and Al ions are just bal-

<sup>3</sup> Coördination number, as applied to crystals, refers to the number of ions bearing a given kind of charge, which immediately surround an oppositely charged ion. In the silicates the positively charged Si ions are always surrounded by four negatively charged O ions, whereas the Al ions of aluminosilicates are surrounded by either four or six O ions. Hence, the coördination number of Si is 4, whereas the coördination number of Al may be either 4 or 6.

anced by the negative charges of the O and OH ions; hence, these minerals contain no bases. However, the OH ions of kaolinite and pyrophyllite are essential parts of the lattice structure. Such minerals are said to be hydrous, that is, water is driven off at high temperatures. Upon complete dehydration a fundamental alteration takes place in their crystal structure, as is shown by X-ray examination.

#### CONDITIONS ESSENTIAL TO ION EXCHANGE

The two conditions which we consider essential to base exchange are; first, physical accessibility of the ion and, second, the strength of the attractive force by which the ion is held to the particle, that is, to the lattice. Further discussion of these points will be given later.

#### EXPERIMENTAL RESULTS

*Feldspars.* The feldspars are composed of so-called linked tetrahedra, consisting of both Si tetrahedra and Al tetrahedra. As is well known, the feldspars contain one equivalent of K, Na, or Ca for each Al atom. Since the Al tetrahedra contain, on the average, one excess negative charge, this is compensated for in orthoclase by the intake of a corresponding number of K ions at the time when the crystals are formed. Potassium ions are essential to the structure of orthoclase, but they are replaceable when brought into exposure by grinding. Similar statements can probably be made concerning Na in albite, Ca in anorthite, and Ca and Na in the soda-lime feldspars.

Table 1 gives the base-exchange capacities of several feldspars ground to different degrees of fineness. It will be noted that the exchange capacity, as measured by the neutral  $\text{NH}_4$ -acetate method, was increased markedly by grinding in a ball mill, especially in the case of orthoclase and albite. Unfortunately, we are not at present able to state the exact particle sizes of the samples used in this investigation, but it is certain that the average size of the particles was substantially reduced by the grinding process in the case of every mineral studied. The base-exchange capacities of the different feldspars, as reported in table 1, are not strictly comparable, because the different kinds of feldspars were ground under different conditions; but the different periods of grinding for a given feldspar, orthoclase for example, are strictly comparable. A rubber-lined ball mill and polished agate balls were used in all the grinding experiments. Therefore, the possibility of contamination of the samples through abrasion of ball mill parts was limited to silica.

*Zeolites and permutites.* As is well known, both the natural zeolites and the artificial permutites have high base-exchange capacity (see table 2). The Al ion of the zeolites is tetrahedral, and the same is probably true of permutite; consequently, these substances must contain additional cations (bases). In the zeolites the ordinary bases are located on exposed surfaces because these minerals have a honeycombed structure. The bases are arranged on the surface of intercommunicating internal cavities. These channels permit the

passage of ions, and therefore base exchange is physically possible on the interior of the crystals. Hence it is not necessary to reduce the size of the crystals of natural zeolites to extreme fineness in order to replace the bases. For example, we have found that practically all of the Na of analcite, a mineral related to the zeolites, can be replaced by K, as KCl, without excessive grind-

TABLE 1  
*Base-exchange capacity of feldspars*

		<i>m.e. per 100 gm.</i>
Orthoclase.....	100 mesh	5.0
	Ground 24 hours	22.0
	Ground 48 hours	42.0
	Ground 120 hours	91.5
Albite.....	100 mesh	1.0
	Ground 48 hours	54.0
	Ground 72 hours	70.0
Labradorite.....	100 mesh	2.0
	Ground 72 hours	26.0
	Ground 96 hours	37.0
Oligoclase.....	100 mesh	2.6
	Ground 72 hours	27.0
Anorthite.....	100 mesh	2.0
	Ground 72 hours	24.0
	Ground 96 hours	33.0

TABLE 2  
*Base-exchange capacity of zeolites and permutite*

		<i>m.e. per 100 gm.</i>
<b>Zeolites:</b>		
Scolecite.....	Ground moderately	209.3
Stilbite.....	Ground moderately	304.5
Natrolite.....	Ground 48 hours	74.5
Natrolite.....	Ground 72 hours	108.5
Permutite.....	Unground	225.0
	Ground 48 hours	225.0

ing of the sample. When this replacement has been effected, the product is an entirely different mineral, namely, leucite. This is in agreement with Lemberg's results.

On the other hand, permutite is commonly held to be amorphous; its specific surface is high. Consequently, its base constituents are accessible to the cations of solutions and are replaceable.

Thus the bases of zeolites and permutites conform to the first condition for replaceability, mentioned previously. Jaeger (7) has given a diagrammatic representation of the crystal structure of ultramarine, a mineral that is related to the zeolites, in which he showed the replaceable Na ions and H<sub>2</sub>O molecules on the interior surface of the crystal.

The zeolites represent very much more open packing of the tetrahedral ionic clusters than do the feldspars. The latter are, accordingly, much harder minerals, and, in turn, much more drastic grinding is necessary to bring the bases into exposure.

Pyrophyllite		Talc	
6O <sup>-</sup>		6O <sup>-</sup>	
4 Si <sup>+</sup> <sup>4</sup>		4 Si <sup>+</sup> <sup>4</sup>	
4O <sup>-</sup> + 2OH <sup>-</sup>		4O <sup>-</sup> + 2OH <sup>-</sup>	
4Al <sup>+</sup> <sup>3</sup>		6Mg <sup>++</sup>	
4O <sup>-</sup> + 2OH <sup>-</sup>		4O <sup>-</sup> + 2OH <sup>-</sup>	
4Si <sup>+</sup> <sup>4</sup>		4Si <sup>+</sup> <sup>4</sup>	
6O <sup>-</sup>		6O <sup>-</sup>	
Muscovite		Biotite	
6O <sup>-</sup>		6O <sup>-</sup>	
3Si <sup>+</sup> <sup>4</sup> + Al <sup>+</sup> <sup>3</sup>		3Si <sup>+</sup> <sup>4</sup> + Al <sup>+</sup> <sup>3</sup>	
4O <sup>-</sup> + 2(OH <sup>-</sup> , F <sup>-</sup> )		4O <sup>-</sup> + 2(OH <sup>-</sup> , F <sup>-</sup> )	
Origin 4Al <sup>+</sup> <sup>3</sup>	Charge -2	6Mg <sup>++</sup>	Charge -2
4O <sup>-</sup> + 2(OH <sup>-</sup> , F <sup>-</sup> )		4O <sup>-</sup> + 2(OH <sup>-</sup> , F <sup>-</sup> )	
3Si <sup>+</sup> <sup>4</sup> + Al <sup>+</sup> <sup>3</sup>		3Si <sup>+</sup> <sup>4</sup> + Al <sup>+</sup> <sup>3</sup>	
6O <sup>-</sup>		6O <sup>-</sup>	
2K <sup>+</sup>	} +2	2K <sup>+</sup>	} +2
6O <sup>-</sup>		6O <sup>-</sup>	
3Si <sup>+</sup> <sup>4</sup> , etc.	} -2	3Si <sup>+</sup> <sup>4</sup> , etc.	} -2

FIG. 1. SEQUENCE OF ATOM-PLANES ALONG THE PSEUDO-HEXAGONAL AXIS OF PYROPHYLLITE, TALC, MUSCOVITE, AND BIOTITE  
Reproduced from Pauling (15)

*The micas.* As is well known, the micas are platy minerals. They cleave most readily along one axis. According to Pauling, (15) the crystal structure of the micas is made up of a series of planes of ions as shown in figure 1. It will be noted that the K ions all occur on the cleavage planes. It follows then that, were it possible to separate the sheets of mica down to the thickness of a single lattice package, all of the K ions would be exposed. Grinding tends to do this; hence it increases the base-exchange capacity, as shown in table 3.

Biotite contains Mg ions as essential lattice constituents. According to Pauling, the Mg ions of biotite bear the same relation to O and OH ions as the octahedral layer of Al ions in muscovite (see figure 1). Moreover, the Mg-

containing octahedra of biotite are similar to those of talc and brucite. When finely ground, is this Mg replaceable? We have found that relatively much Mg can be extracted from finely ground biotite by neutral  $\text{NH}_4$  salt solutions, but that the amount of  $\text{NH}_4$  absorbed from the salt solution is equivalent only to the K that is removed from the sample (see table 4). Further reference to this point will be made in connection with the discussion on talc.

*Talc.* According to Pauling, (15) each Mg ion in talc is surrounded by four O and two OH ions. The layers of lattices are held together by stray electrical forces, as contrasted with muscovite and biotite in which the layers are held together by K ions (see figure 1). Talc is a relatively soft mineral and is

TABLE 3  
*Base-exchange capacity of the micas*

		<i>m.e. per 100 gm.</i>
Muscovite.....	100 mesh	10.5
	Ground 72 hours	76.0
Biotite.....	100 mesh	3.0
	Ground 48 hours	62.0
	Ground 72 hours	72.5

TABLE 4  
*Effect of ammonium acetate on the micas*

		BASES EXTRACTED BY $\text{NH}_4$ ACETATE		ABSORBED
		Mg	K	$\text{NH}_4$
		<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
Muscovite.....	100 mesh	Tr.	9.1	10.5
	Ground	Tr.	52.5	48.3
Biotite.....	100 mesh	14.3	8.2	8.8
	Ground	113.0	60.0	62.0

readily ground to extreme fineness. When it is thus ground, we have found that  $\text{NH}_4$  salt solutions are able to extract large amounts of Mg, and at the same time the substance appears to undergo decomposition. This decomposition may be viewed in different ways: First, we may assume that  $\text{NH}_4$  ions replace those Mg ions which are present on the surface of broken octahedra, with the resulting formation of  $\text{NH}_4$  silicate, which is an unstable compound. Or, second, we may say that the  $\text{NH}_4$  ions first replace Mg ions, and then the  $\text{NH}_4$  ions are replaced by H ions of the solution, thus converting the substance into silicic acid; this, of course, is equivalent to saying that  $\text{NH}_4$  silicate hydrolyzes. Or, third, we may look upon the case as follows: When  $\text{NH}_4$  replaces the octahedral Mg ions, the  $\text{NH}_4$  ions find themselves attached to O and OH ions,

which is an unstable arrangement. Whatever be the correct explanation, the fact that the Mg of biotite and talc behaves similarly toward  $\text{NH}_4$  salt solutions is in harmony with Pauling's conclusions regarding the similarity in the crystal structure of these minerals.

*The chlorites, pyroxenes, and amphiboles.* Pauling (16) has shown that, from a crystal structure standpoint, the basic igneous minerals are combina-

TABLE 5  
*The effect of ammonium acetate on talc, chlorite, bauxite, and quartz*

		BASES EXTRACTED BY $\text{NH}_4$ ACETATE		ABSORBED
		Ca	Mg	$\text{NH}_4$
		<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
Talc.....	100 mesh	Tr.	9.8	Tr.
	Ground	Tr.	219.0	Tr.
Chlorite.....	100 mesh	5.0	32.0	6.0
	Ground	35.0	88.0	34.5
Prochlorite.....	60 mesh	Tr.	12.5	Tr.
	Ground	Tr.	243.0	50.0
Bauxite.....	100 mesh	Tr.	Tr.	Tr.
	Ground	Tr.	Tr.	Tr.
Quartz. ....	100 mesh	0	0	Tr.
	Ground	0	0	2.0

TABLE 6  
*Base-exchange capacity of kaolinite and pyrophyllite*

		<i>m.e. per 100 gm.</i>
Kaolinite.....	100 mesh	8.0
	Ground 48 hours	57.5
	Ground 72 hours	70.4
	Ground 7 days	100.5
Pyrophyllite.....	100 mesh	4.0
	Ground 48 hours	72.5
	Ground 72 hours	92.0
	Ground 7 days	158.5

tions of the structures of mica and brucite. Since Mg in the brucite-like octahedral arrangement splits off upon treatment with  $\text{NH}_4$  salt solution without the absorption of  $\text{NH}_4$ , and since Mg is the predominant base of these minerals, their base-exchange capacities, as measured by the ammonium acetate method, should be relatively low (see table 5).

*Pyrophyllite and kaolinite.* Since pyrophyllite and kaolinite contain none

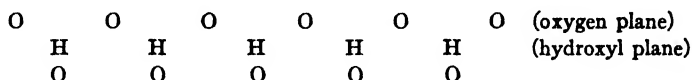


of the common bases, it is especially interesting to find that, upon being ground to extreme fineness, they possess high cation-exchange power (see table 6). We believe that this exchange power is traceable to the H ions of the crystal lattice of these minerals.<sup>4</sup>

From the standpoint of H-ion replacement two types of OH planes are of special significance. First, a plane of OH ions immediately on the exposed surface of a lattice package which is arranged in the following manner:



Second, a subsurface OH plane, which is covered by a network of O ions, as illustrated by the following:



The important feature of the second type of arrangement lies in the fact that the H ions of the OH plane are accessible through the meshes of the superimposed O network. Each of the two surfaces of pyrophyllite lattice packages consists of the latter type of plane, whereas kaolinite contains both types. Just how readily these H ions can be replaced is not known at present; one would expect, however, that they are held rather tightly and that alkaline solutions would be required to produce efficient exchange.

Grinding may split these crystals parallel to the basal planes or vertical to them. In the first case new planes containing H ions would become exposed; in the second case the octahedral groups of the lattice (10, fig. 14) would be broken and the bond strength between O and H be altered.

*The bentonites and soil colloids.* The published analyses of bentonite always show more or less Ca and Mg and usually small amounts of Na and K. In our experience, practically all the Ca, K, and Na of the more common types of bentonitic clays are replaceable, provided the necessary steps are taken to separate the clay constituents from fragments of associated minerals. On the other hand, only a minor percentage of the Mg is replaceable, but additional amounts of Mg become either replaceable or extractable after the mineral has been ground in a ball mill. As Kelley, Dore, and Brown (9) pointed out, and as shown in table 7, the base-exchange capacity of a given bentonite, and also of soil colloids, can be markedly increased by grinding the sample.

*Exchange power of bentonites and soil colloids.* At present we cannot say what is the upper limit of the base-exchange capacity of the bentonitic clays and soil colloids. We have been able to raise the base-exchange capacity of one sample of bentonite from 126 to 238 m.e. per 100 gm., and it is highly probable that further grinding would produce still further increase. With one Yolo

<sup>4</sup> Direct experimental proof that the exchange power of ground kaolinite and pyrophyllite is due to H ions of the lattice will be published elsewhere.

soil colloid we have raised the exchange capacity from 67 to 166 m.e. In the case of the Cecil colloid the capacity was increased from 17 to 151 m.e. and the Redding soil colloid was affected similarly. With the Yolo colloid the increased exchange capacity appears to be due chiefly to Mg and K ions, whereas in the Cecil and the Redding it was occasioned mainly by H ions brought into exposure by the grinding process.

TABLE 7

*Base-exchange capacity and pH of bentonite, beidellite, talc, and soil colloids as affected by grinding\**

		<i>m.e. per 100 gm.</i>	<i>pH</i>
Bentonite No. 2.....	1 micron	126.0	7.3
	Ground 72 hours	238.0	9.1
Beidellite.....	1 micron	50.0	6.4
	Ground 48 hours	200.5	7.6
Talc.....	100 mesh	Tr.	6.9
	Ground 48 hours	Tr.	8.9
Yolo soil colloid.....	1 micron	67.0	7.3
	Ground 48 hours	166.0	7.3
Cecil soil colloid.....	1 micron	17.0	7.2
	Ground 48 hours	151.0	5.4
Redding soil colloid.....	1 micron	35.0	6.6
	Ground 48 hours	122.5	5.5

\* Before being subjected to the grinding process, the samples of bentonite, beidellite, and soil colloids were Ca saturated by leaching with neutral Ca acetate solution.

#### ON THE NATURE AND STRENGTH OF ATTRACTION FORCES

Goldschmidt and Pauling have shown that it is possible to explain many of the features of aluminosilicates by assuming that the atoms are electrically charged spheres (ions) and that they are held together with electrostatic forces of the Coulomb type. We believe that the same forces are active in base-exchange reactions, especially if ions of large size, such as K, Cs, or hydrated Li and Na, are involved. For some ions, such as the H ion, and possibly Be and Mg ions also, a transition to electron-pair bonds appears plausible.

When lattice ions become exposed on the surface, they experience greater freedom of movement and oscillate over greater distances; thus they may be replaced by other ions according to the mechanism proposed by Jenny (8). The term "surface dissociation of ions," which is frequently used in soil literature, does not appear appropriate, except perhaps for the H ion, because the bases are considered to be already dissociated in the solid state.

The behavior of the H ion is particularly interesting. According to our

viewpoint it lies opposite an O ion (in exceptional cases opposite an OH ion) in such systems as are represented by hydrous, crystallized aluminosilicates. The feldspars do not contain OH ions, but upon hydrolysis or electrodialysis the K, Na, or Ca ions become replaced by H ions, and then H ions are attached to O ions. This viewpoint leads directly to a parallelism with many organic substances; proteins, for example, also show base exchange and they also have

an exchangeable H ion associated with an O ion ( $\text{C}=\text{O}-\text{OH}$  group). The activity of these exchangeable H ions depends upon the intensity of the attraction between H and O, which in turn is influenced by the neighboring lattice ions. We believe that the different acidities of many soil colloids and minerals can ultimately be explained by the nature of the attractive forces acting on various parts of the crystal lattice, such as the planes, corners, or edges.

#### DISCUSSION

From the foregoing it follows that the base-exchange power of crystalline minerals may be explained on the basis, first, of accessibility and, second, of the strength of the attractive force by which the bases are held to the lattice. More precisely, it may be said that, with the exception of the bases that have replaced H ions from the broken lattices of the hydrous minerals, only those metallic cations which perform the function of balancing the excess negative charges of the tetrahedral groups (and possibly the negative charge of the octahedra in exceptional cases) are reversibly replaceable by  $\text{NH}_4$ . With the platy minerals of the mica group, these metallic cations occur on the surface of the cleavage planes; in the zeolites and feldspars they occur on the surface of internal cavities. These cavities are large enough in the zeolites to permit access to the ions of a solution, whereas with the feldspars the structure is more compact; the bases are similarly situated in the lattice of both types of minerals.

Although we have not as yet investigated all the various classes of silicates, it seems probable that the K and the Na of all of them are essentially replaceable, provided the particles can be made small enough to permit physical access to these ions. The same may be true of Ca, but, as has been indicated, the case is considerably more complex for Mg. The octahedral Mg is probably not stoichiometrically replaceable by the alkalis or  $\text{NH}_4$ , at least not when OH ions constitute parts of the octahedra. On the other hand, Mg ions can readily replace other replaceable cations, and the process is known to be reversible.

Recently, van der Meulen (13) pointed out that the various silicates may be subdivided into three different groups: First, those containing no Al; second, those containing Al with coordination 6; and third, those containing Al with coordination 4. He claims that the first two groups have practically no base-exchange property but that the third group has definite base-exchange property. In the group which contains no Al there are such well-known minerals as

quartz, talc, and wollastonite. In the group containing Al with coördination 6, van der Meulen lists kaolinite and cyanite. He holds that the cation-exchange power of these minerals is practically zero. However, to his list we must add pyrophyllite and probably montmorillonite and beidellite; but the last two of these have extremely high base-exchange power in their natural condition, and the same is true of finely ground kaolinite and pyrophyllite. As minerals the aluminum of which has a coördination 4, van der Meulen names several zeolites, and also orthoclase and other feldspars, the micas, and several other minerals.

Van der Meulen points out that, although the open structure of the zeolites makes possible an exchange of cations on the interior of the crystals, this is not true of a feldspar crystal. He states, "If, however, the crystal is very finely pulverized a great number of Al atoms with coördination value 4 are at the surface of the particle and become accessible for exchanging cations." Although careful reading of van der Meulen's paper shows that his view is essentially the same as ours, in so far as the exchange property of the feldspars and zeolites is concerned, the statement just quoted is inaccurately phrased, because it attributes the cation-exchange power to the exposed Al atoms themselves. It is more correct to say that the negative charges imparted to the lattice by virtue of tetrahedral Al ions are responsible for the fact that orthoclase and the micas *contain* K ions and that these K ions are replaceable upon exposure. The K ions are directly attached to O ions of the Al tetrahedra in the zeolites, orthoclase, and the micas.

When kaolinite and pyrophyllite are finely ground, relatively many OH ions of the lattice become exposed because breaks are produced either across the octahedral layers or parallel thereto. We believe that the cation-exchange power of these ground minerals is due in large part, at least, to the OH groups that are exposed by breaks across the octahedral layers, but it must be admitted that as yet we have been unable to prove definitely just where the active exchange spots are located. We have found, in agreement with other investigators, that relatively coarse particles of these minerals (100 mesh) have only low cation-exchange power (4 to 8 m.e.). Even in this case the cation-exchange power is probably due to H ions on the surface of broken lattices of the crystals.

Hofmann, Endell, and Wilm (5) have published a diagram, reproduced as figure 2, that is intended to show the seat of the exchangeable ions on a clay crystal. According to this model, the cations are held by broken bonds on the edges of the Si-O planes. At first sight this viewpoint is very appealing, but upon closer examination a serious difficulty arises. Suppose we take a clay crystal containing an extensive Si-O plane, and break it, in the air, into a large number of small pieces. Tetrahedra will be broken and many free bonds will necessarily appear on the broken surfaces, but, up to this point, no replaceable bases will be present, since there were none in the original material. Hofmann, et al. hold, it is presumed, that these are adsorbed by the free val-

ences when the crystal fragments come into contact with a solution containing cations (K, Ca, H, etc.). It is just here that the difficulty comes in. If the broken bonds should bind cations from the solution, what would happen to the remaining anions in the solution? If their hypothesis is valid, then when the clay fragments were removed from the solution more anions than cations would be left in the solution, but such a system cannot exist, for it violates the law of electrical neutrality. In other words, a solid cannot adsorb cations from solutions without an exchange process or an equivalent adsorption of anions. The model of Hofmann, et al. (fig. 2) does not satisfy either of these two conditions.

Theoretically, the following modification would overcome the objection raised. The broken bonds might polarize and adsorb water molecules, which dissociate on the clay surface into OH and H ions. The latter then act as

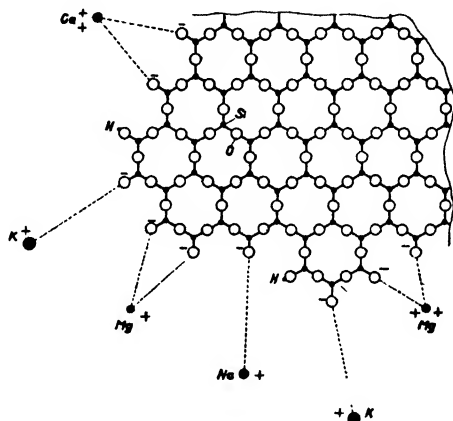


FIG. 2. DIAGRAMMATIC REPRESENTATION OF A Si-O LAYER OF A CLAY CRYSTAL WITH CATIONS ATTACHED TO THE FREE O VALENCES ON THE EDGES OF THE BROKEN LATTICES

Reproduced from Hofmann et al. (5)

replaceable cations. Experiments to test this hypothesis, however, have thus far given practically negative results (see the data for quartz, table 5). At present we must conclude that the bulk of the exchangeable cations of the clay minerals are not held by the broken bonds on the edges of the Si-O planes.

Since the bentonitic clays contain OH ions as lattice constituents and since their individual particles are extremely small, we believe that, with the possible exception to be noted presently, the cation-exchange power of the natural bentonites is due to exposed OH groups of the crystal lattice. With natural bentonites the H ions of these exposed OH groups have already been replaced in the state of nature, and for this reason the bentonites are usually saturated with exchangeable metallic cations. Upon grinding, additional OH groups become exposed, and thus the exchange capacity is increased.

Hofmann, et al. (5) and Marshall (12) have suggested that Mg and Fe may

occupy a position in the bentonite (montmorillonite-like) crystals, similar to that of octahedral Al, that is, surrounded by O and OH ions (10, fig. 14). As Marshall (12) suggests, this would either produce a mixed hydrargillite-brucite structure, in which case the lattice would acquire one negative charge for each Mg ion thus introduced and therefore additional cations would be required to balance these negative charges, or else it would produce a brucite-like structure.

Either of these alternate modes of substitution of Mg for Al would introduce Mg ions into the octahedral positions. Upon grinding material of this kind, it is reasonable to suppose that more or less of such Mg ions would become exposed, as in the case of ground biotite and talc, but these Mg ions ought not to be stoichiometrically replaceable by  $\text{NH}_4$ . However, they should be extractable by  $\text{NH}_4$  salt solutions. It is interesting to note that the pH of bentonite is affected by grinding (see table 7) very much as is talc, a mineral the Mg of which certainly occupies the octahedral position. It is probable that the Mg ions, brought into exposure by breaks across the octahedra, hydrolize and thus produce increases in the pH.

On the basis of data obtained with samples of bentonitic clays from several different localities in North America, there appears to be some relation between content of nonreplaceable Mg and base-exchange capacity. For example, Vanselow has found that Wyoming bentonite from the type locality contains approximately 1.6 per cent nonreplaceable MgO and has a base-exchange capacity of 86 to 88 m.e. per 100 gm. On the other hand, another type of bentonite obtained from two different localities, namely, Otay, California, and Goldfield, Nevada, contains about 4.60 per cent nonreplaceable MgO and has a base-exchange capacity of about 107 m.e.<sup>5</sup> These differences in base-exchange capacity are less than one-third what they should be if each Mg ion imparts a negative charge to the exposed surface of the particles.

Marshall (12) has also concluded that Al can replace Si in the lattice of the clay crystals, as well as be replaced by Mg, Fe, and Ti. Should one-fourth of the Si ions be replaced by Al ions, this would produce a structure closely analogous to that of muscovite minus its K ions (assuming that the ideal structure of montmorillonite by Hofmann, et al. is essentially correct). The substitution of Al for Si, however, would certainly result in one negative charge on the lattice for each Si ion that is replaced. Just as in the micas, these negative charges would have to be neutralized by the absorption of cations, and such cations might be held on the interplanar surfaces of the lattice. Wherever they occur, whether on the surface of the lattice framework or on its interior, these cations would be replaceable upon exposure.

It is possible that the substitution of Al for Si is involved in certain Yolc soil colloids of California but that the replacement of Al by Mg plays an insignificant part in them. We have found, for example, that when a sample of Ca-

<sup>5</sup> The authors are grateful to Dr. Vanselow for these data.

saturated Yolo colloid is ground, additional Mg and K ions become replaceable, but that the pH is not materially altered, indicating that this colloid does not contain significant amounts of octahedral Mg. Preliminary X-ray examination of this colloid has failed to show any relation between the content of adsorbed water and the X-ray spectrograph. In this respect it appears to be similar to the unidentified clay mineral discussed by Jacob, et al. (6).

The ideas developed in the foregoing paragraphs suggest that the bentonitic types of clays are structurally related to the micas, and it is interesting to note that their properties are similar in several respects. It must be admitted, however, that the structure, as proposed by Hofmann, et al. is only a first approximation. Further investigation may necessitate modifications in their lattice structure. It is important to bear in mind that the determination of the structure of fine-grained substances, like the bentonitic and other clays, is difficult. Only powder X-ray spectrographs of these minerals have as yet been made and these are commonly difficult to interpret.

In collaboration with Mr. W. H. Dore, we have verified the conclusion of Hofmann, et al. (4), that the interplanar distances between lattice packages of the bentonitic clays vary according to water content. More recently Jacobs, et al. (6) have found that certain soils contain minerals similar to kaolinite, other soils contain minerals similar to montmorillonite, and still others contain an undetermined crystalline clay mineral. These conclusions are in harmony with those of Marshall (12) which were based on calculated substitutions within the lattice of different samples. They also agree with the dehydration data of Kelley, Jenny, and Brown (10) and with unpublished X-ray data obtained in connection with this investigation.

Reference again to the effect on pH, produced by grinding (table 7), will show that, whereas Ca-saturated bentonite and talc become more alkaline when ground, the Ca-saturated Cecil and Redding soil colloids become more acid. On the other hand, the pH of Yolo soil colloid is altered but little by grinding, whereas that of beidellite is increased by grinding to an intermediate degree. When these facts are considered in conjunction with (a) the dehydration curves of these materials (10), (b) the unpublished X-ray and chemical analyses, and (c) the data showing the effect of grinding on the content of replaceable Ca, Mg, K, Na, and H ions, it is difficult to avoid the conclusion that the Yolo colloid is more like beidellite than montmorillonite, though not identical with beidellite, and that the Cecil and Redding colloids are utterly different from either montmorillonite or beidellite, in fact, they seem to resemble the kaolinitic minerals.

It is highly probable that soil clays embrace a complex series of isomorphous minerals and that certain soils contain one general type of clay mineral, whereas other soils contain a second or a third type. The type actually predominating in a given mature soil would be expected to be that which is most stable under the conditions prevailing in that soil. On the other hand, it seems reasonable to suppose that immature soils might contain variable mixtures of clay minerals. At any rate, all of these clay minerals have more or less base-exchange power.

It follows, then, that no one single substance is responsible for base exchange in all soils. Jacob, et al. (6) have concluded that the base-exchange property of soils is not due to zeolites or permutite-like substances. Kelley, Dore, and Brown (9) drew similar conclusions in 1931.

Finally, we wish to emphasize the fact that the authors consider the views developed in this paper to be essentially tentative. We believe that the OH of the lattice of clay minerals is an important source of their potential cation-exchange power, but it does not follow that the OH group is the sole cause of their exchange power. Should further investigation definitely establish that Al replaces Si in the clay minerals, and also that Mg replaces more or less Al, the negative charges thus introduced on the lattice would certainly necessitate the introduction of cations, and such cations would probably be potentially replaceable. As Marshall has pointed out, there are several other possible substitutions which would also influence the exchange power. In the present state of knowledge it is not possible to decide definitely between these possibilities.<sup>6</sup>

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# STUDIES ON PODZOLS AND BROWN FOREST SOILS: III

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The first part of this paper dealt with the acid-oxalate method of Tamm, used to demonstrate the differences between podzols and brown forest soils (4). The second part showed that the amphoteric properties of the soil colloids may be used for studies of the soil forming processes and that they give reliable information about the differences between the soil types investigated (5). It will now be shown that dye adsorption can also be used as a means of characterization. The profiles studied are the same as those used in the previous parts of the paper (4, p. 146-148).

## ADSORPTION OF ACID AND BASIC DYES

It has long been known that colloids are able to "adsorb" organic dyes of different kinds. Many of the common color reactions used in microscopical work are based on this ability.

The first attempts to determine the amounts of colloids in soils through their adsorption of dyes seem to have been made by Endell and by Ashley. Endell's method—here quoted from Hissink—was very similar to the common way of dyeing microscopical preparations (2, p. 38). Ashley's method—here quoted from Stremme—is colorimetric and similar to the one now employed by the author (9, p. 26). Aarnio and Stremme found that methylene blue was a suitable dye but that the other dye solutions tried were discolored in the presence of lime carbonate (9; 10, p. 27).

The adsorption of methylene blue has since been used by several investigators. A real explanation of the reaction between dye and colloid was first given by Mattson (6, 7, 8). Stremme anticipated the conception of the soil colloids as ampholytoids when writing about the methylene blue adsorption: "In Böden mit saurer Reaktion könnten Tonerde und Eisenoxyd eher als Basen wirken während sie in Böden mit alkalischer Reaktion eher Säuren sein könnten" (9, p. 31). Mattson's results concerning the adsorption of methylene blue can be summarized as follows (8, p. 381): The methylene blue cation is completely adsorbed over the entire range on the electronegative side of the isoelectric point; therefore, the quantity adsorbed is directly proportional to the amount of colloid when the resulting complex is isoelectric. The quantities thus adsorbed by different colloids are a direct measure of their exchange capacity. The methylene blue cation displaces other cations but is not dissociated by the particles; therefore, the complex is isoelectric when the other cations are displaced. The adsorption proceeds beyond the isoelectric point but becomes

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<sup>1</sup> The laboratory work involved in this study was done by the author in the laboratory of Dr. Sante Mattson during a stay as Cook-Voorhees Research Fellow at the New Jersey Agricultural Experiment Station October, 1931-September, 1932 [see acknowledgment in the first part of this paper (4)].

more and more incomplete as the positive charge increases. The fact that this dye is totally adsorbed up to the isoelectric point suggests a possibility of studying the soil colloids both qualitatively and quantitatively.

If the theory of isoelectric weathering is true we should expect a high adsorbing capacity for methylene blue in the  $A_2$  horizon of a soil of the podzol type because the acid weathering should leave there a colloid complex rich in more or less strong acidoid groups. The adsorbing capacity should then decrease through the B and C horizons where colloids richer in ampholytoid groups, such as sesquioxides, and poorer in acidoid groups have been deposited as a result of the weathering. But the adsorbing capacity—though in the strict sense of the word a qualitative property—depends also on the quantity of colloids present, and if the percentage of colloids is high the adsorbed quantity of dye can be larger than that in a soil of a relatively much stronger acidoid character but poorer in colloidal material.

The basic dye night blue was used for some of the experiments though later it was abandoned for methylene blue because of some difficulties in its use. In the experiments with cataphoresis in night blue solutions, the measurements were made in the way previously described. The results are shown in table 7.

From the results it is apparent that the total adsorption continues to the isoelectric point—the fact that, in some cases, a slight coloration is observed at low concentrations of the dye is accounted for by an incomplete flocculation. On the other side of the isoelectric point additional dye is adsorbed, as found from other experiments, but the percentage of dye taken from the solution decreases with increasing concentrations until a point is reached where an increase in concentration does not materially affect the adsorption. The amounts of dye adsorbed at the isoelectric point are (partly interpolated values) in  $A_2$ , 0.90; in  $B_1$ , 1.30; in  $B_2$ , 0.34; and in  $C_2$ , 0.13 mgm. per gram of soil. Thus we find a higher adsorption in  $B_1$  than in  $A_2$  though the acidoid character of the colloids must be more pronounced in  $A_2$ . This means that the quantities of colloids present veil their qualitative properties. The pH values of the different isoelectric systems were about as follows:  $A_2$ , pH 4.5;  $B_1$ , pH 4.7;  $B_2$ , pH 5.0; and  $C_2$ , pH 5.3. The quantities of dye required to render the soils from the various horizons isoelectric are, with the exception of  $B_1$ , very closely proportional to their combining capacities at pH 7, as given in table 6 and in figure 10 of part II (5). The fact that the value of 1.30 mgm. in the case of  $B_1$  is, in proportion to the combining capacity, so much lower than the other values may be accounted for by the relatively low pH of 4.7. At this pH the strongly basoid  $B_1$  cannot be expected to combine with very many cations, even those of a basic dyestuff.

This experiment shows that adsorption of basic dyes may possibly give a false impression of the quality of the colloids in a soil profile and therefore no certain idea of the type of soil forming process, just as determinations of the base exchange only are not conclusive. It was previously suggested that

the base exchange determinations be combined with determinations of the anion exchange capacity. Just as this property would give true information about the soil forming process in the case of soils formed by the acidoid type of weathering, so it is quite natural that an acid dye would give the same re-

TABLE 7

*Cataphoresis of soil samples from a podzol profile, number 5, in night blue solution*  
2.5 gm. of soil in 25 cc., measurements after 24 hours

NIGHT BLUE	CATAPHORESIS	COLOR OF SOLUTION	CATAPHORESIS	COLOR OF SOLUTION
	A <sub>2</sub> horizon		B <sub>1</sub> horizon	
mgm.	$\mu/\text{sec.},$ 1 volt/cm.		$\mu/\text{sec.},$ 1 volt/cm.	
0	-3.19		-1.26	
0.25	-3.03	Very light blue	-1.08	Nearly colorless
0.50	-2.75	Very light blue	.....	
0.75	-2.52	Nearly colorless	-1.08	Colorless
1.00	-2.47	Nearly colorless	.....	
1.25	-2.33	Colorless	.....	
1.50	-2.02	Colorless	.....	
1.75	-0.95	Colorless	-1.01	Colorless
2.00	-0.38	Colorless	-0.87	Colorless
2.25	- slight	Colorless	-0.67	Colorless
2.50	+1.51	Blue	.....	
2.75	.....		-0.43	Colorless
3.00	+2.52	Blue	-0.38	Colorless
3.25	.....		$\pm 0.00$	Colorless
3.50	.....		+0.38	Very light blue
4.00	+2.63	Blue	.....	
5.00	+3.03	Blue	+1.26	Blue
	B <sub>2</sub> horizon		C <sub>2</sub> horizon	
0	-1.29		-1.44	
0.25	-0.84	Colorless	-0.30	Colorless
0.50	.....		+0.87	Very light blue
0.75	-0.34	Colorless	+1.21	Light blue
1.00	+0.50	Nearly colorless	+2.33	Blue
1.25	+1.21	Very light blue	+3.03	Blue
1.75	+1.26	Light blue	+3.37	Blue
2.25	+2.02	Blue	+3.79	Blue
3.25	+2.42	Blue	+4.04	Blue
5.00	+3.03	Blue	+4.67	Blue

sult. At the same time, as the acid residues in the colloid complexes of these soils decrease in strength—but, as pointed out, not necessarily in amount—with increasing depth, the basic residues increase, not only in strength but also in amount, at least to the B horizon. We would expect, therefore, to

find a low adsorption of acid dyes in the A horizon and a high one in B. A decline in C is possible because of lower amounts of colloids present. From a theoretical standpoint we should not expect to find a complete adsorption of the acid dye on the electronegative side of the isoelectric point but, on the contrary, possibly on the electropositive side. It was also proved experimentally with the soils used for the cataphoretic experiments with night blue that increasing concentrations of an acid dye—alizarine red R—caused an increasing electronegative velocity of the particles. If a suitable concentration of HCl was added, concentrations of the dye could be found which made the complex isoelectric. It is to be deplored that the dye used in the experiments has so weak a color in acid solutions as to make it impossible to determine colorimetrically if and when the adsorption is complete.

From a scientific standpoint it would naturally be best to determine the highest amount of dye completely adsorbed from a solution. This would give values most characteristic for each soil. But because of the difficulties mentioned with the acid dye, this method was not used except in the experiments just reported. Instead a very simple colorimetric method was applied which, though for apparent reasons not giving absolutely comparable results, makes comparative studies of different soil types very easy and rapid. It was used in the following way. A soil sample was repeatedly shaken for a day with a dye solution strong enough to leave some of the dye unadsorbed. The test tubes were left undisturbed overnight; a part of the clear solutions was taken out with a pipette and diluted to a convenient volume; and the amount of dye was colorimetrically determined in a comparator against standard solutions of the dye. From the amount thus determined and the original volume and concentration the amount adsorbed was calculated. The dyes used were night blue, methylene blue (both basic), and alizarine red R (acid). Night blue was found unsuitable for the purpose because of changes of color at different reactions. Methylene blue, however, was found to have a constant color within the pH range of the soils investigated and far beyond on both sides. Since the strength of color is proportionate to the dilution within reasonable limits, the dye is ideal from the viewpoint of colorimetric determinations. Alizarine red R is red in alkaline solutions and yellow in acid. Its point of color change lies within the pH range of the soils, and therefore the samples taken out for estimation must be treated with alkali or acid to get the full color on either side. The alkali color was found suitable for colorimetric work, since it was proportionate to the dilution and fairly strong. The yellow acid color cannot be used, since it is impossible to determine small differences in dilute solutions.

In order to obtain information about the dye adsorption of colloids with different composition, experiments were made with artificial alumino-silicates.<sup>2</sup>

<sup>2</sup> These were prepared by Dr. J. S. Csiky, who at the same time was making other investigations with them at the New Jersey Agricultural Experiment Station. The author wishes to thank Dr. Csiky for his friendliness in supplying samples of this valuable material.

The aluminosilicates were completely electrodyalyzed, and the composition, here quoted as the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio, was determined by Dr. Csiky. The experiments were made in the following way. Samples of 0.2 gm. of each silicate were shaken in test tubes with 10 cc. of dye solution of different concentrations in the way previously described; the unadsorbed dye was estimated colorimetrically and the adsorbed amounts were calculated. The results are

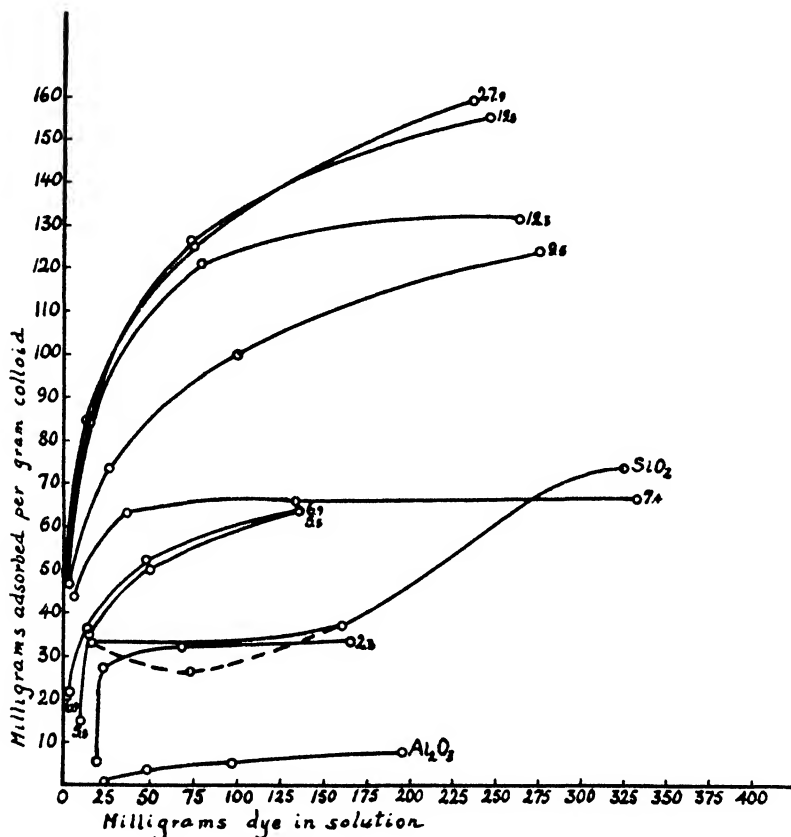


FIG. 13. ADSORPTION OF METHYLENE BLUE FROM SOLUTIONS OF DIFFERENT STRENGTHS BY ARTIFICIAL ALUMINO-SILICATES

The figures at the ends of the curves are the silica/alumina ratios

seen in figures 13, 14 and 15. Objection to figures 13 and 14 may be raised on the ground that three or four points are not enough to permit the drawing of a curve. These curves, however, are not intended to be used for any exact determinations of the process of adsorption but only to show the influence of the composition on the adsorption.

Let us first look at the adsorption curves for methylene blue (fig. 13). It is seen that all the curves (except the  $\text{SiO}_2$  curve) are of the same form, namely,

that of the familiar "adsorption" curves: in low concentrations the dye is proportionally far more extensively adsorbed than in higher concentrations. The amount adsorbed seems to reach asymptotically a certain maximum independent of a further increase in the concentration of the supernatant liquid. This maximum is not reached, at the concentrations used, in the case of alumino-silicates with a very high  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio. The adsorption is higher the wider this ratio, being lowest in the pure aluminum hydroxide, in accordance with the theory the greater the acid residue in the colloid the

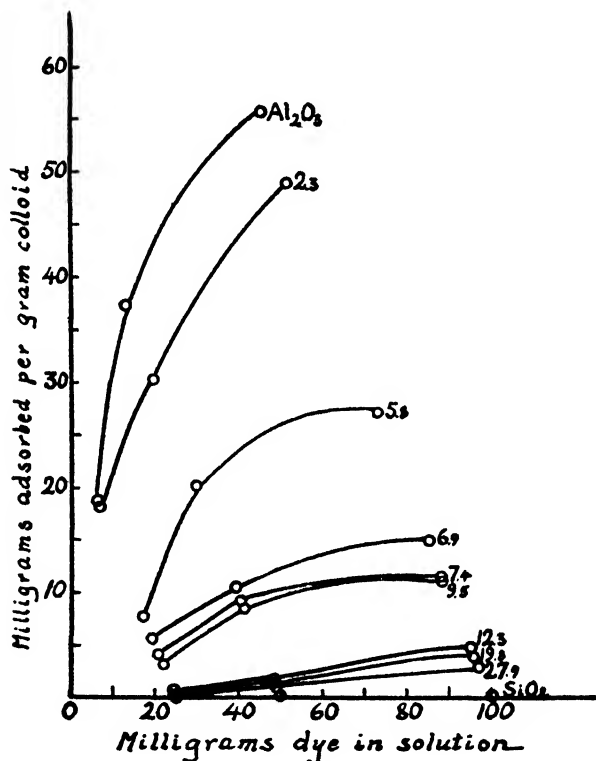


FIG. 14. ADSORPTION OF ALIZARINE RED R FROM SOLUTIONS OF DIFFERENT STRENGTHS BY ARTIFICIAL ALUMINO-SILICATES

The figures at the ends of the curves are the silica/alumina ratios

higher its adsorption for the methylene blue cation. The pure  $\text{SiO}_2$  is an exception: the adsorption is here even lower than that for many of the silicates with a rather narrow  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio. This might depend on the mode of preparation: the silica was not prepared like the silicates (by precipitation from sodium silicate with  $\text{HCl}$  and subsequent electrodialysis) but was a commercial product electrodialyzed for purification. Here we must take into consideration aging of the colloid and various differences in the state of aggregation which might affect the adsorbing capacity. But it is not impossible that the

dye adsorption has a maximum at some wide  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio, just as the base exchange, according to Csiky's results, has a maximum at a certain

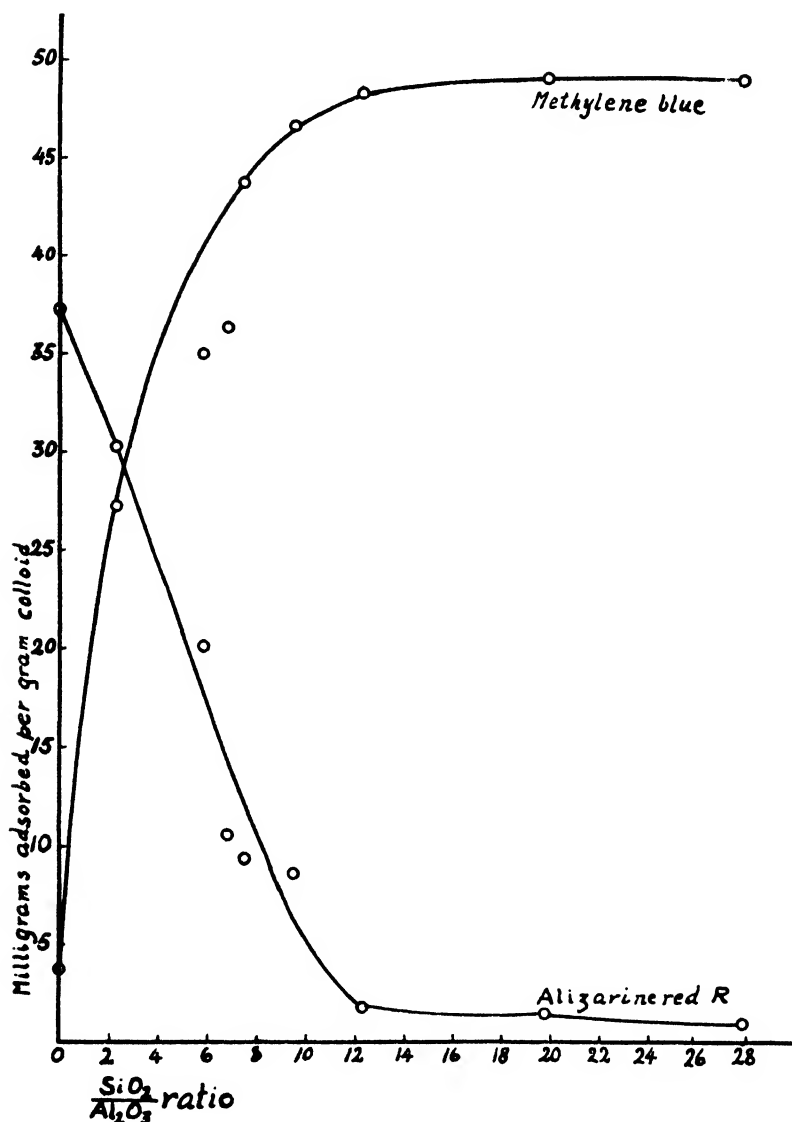


FIG. 15. THE RELATIONSHIP BETWEEN THE COMPOSITION OF ALUMINO-SILICATES AND THEIR ADSORPTION OF METHYLENE BLUE AND ALIZARINE RED R  
50 mgm. of the dye added in each case

composition of the silicate and decreases on both sides of this value. If actually existent, the maximum in the case of the dye adsorption must lie



above the ratio 27.9, i.e., at a much wider ratio than the maximum of base exchange. The reason for this difference might be sought in the low degree of dissociation and hence in the slight hydrolysis of the methylene blue compounds.

The adsorption of alizarine red R gives the same type of curves (fig. 14), the only difference being that in this case the adsorbing capacity decreases with increasing silica/alumina ratio: the more basic the residues in the colloid complex, the higher the adsorption of anions; the more acid the residues, the lower the adsorption of such ions. In this case the pure silica is no exception: it shows no adsorption of the acid dye, just as would be expected with a colloid of pure acidoid character with no perceptible ampholytoid properties. The pure alumina also fits well in the series: it has the highest adsorbing capacity, as would be expected from its ampholytoid nature, for the basic residues should decrease in strength when acidoids like silica are introduced into the complex.

After the work on dye adsorption was completed a paper by F. Hardy on alizarine adsorption of soils, minerals, and artificial alumina and ferric oxide came to hand (1). Hardy does not work with exactly the same dye as here used but with sodium alizarine sulfonate (alizarine S), but its properties seem to be closely related to those of alizarine red R. His method of estimation is also colorimetric, but the dye adsorbed is extracted with a solution of sodium oxalate containing an excess of oxalic acid, the pH being 3.8. This necessitates corrections for the iron dissolved. A still more serious complication would probably arise in the case of soils containing appreciable amounts of organic matter.

Some objections to Hardy's interpretation of his results may be made. The calculations are founded on the assumption that the soils contain the hydrous sesquioxides in a "free" condition. Even if this is the case in lateritic soils formed at reactions within the range of the isoelectric points of the free sesquioxides themselves, there is no proof of the general existence of free sesquioxides in the soil. A multitude of facts concerning the chemical nature of soil colloids tend to show that they are not single chemical compounds occurring side by side, but complexes composed of the acidoids and ampholytoids present, the relative amounts of the components and the type of linking between them depending on the soil forming conditions. The assumption that the "total" adsorption was determined cannot be correct because the adsorption depends on the circumstances. Just as, for instance, the base exchange capacity depends on the pH, the dye adsorption, which is to be considered as an ion exchange, depends on the same quality and thus on the concentration of the solution. This fact is also demonstrated experimentally with the dyes used in the experiments here reported.

These critical remarks on Hardy's work are made only in order to get a truer interpretation of the results. The idea of using the adsorption of dyes as a means of investigating the genesis of soils is very promising, and there-

fore Hardy's work is of great importance. One thing which should be further investigated is the significance of the alizarine adsorption by ignited alumina as against the nonadsorption by some of its natural hydrated compounds.

Let us now return to our soil profiles. Two 2-gm. samples (calculated as oven-dry material) of each soil were repeatedly shaken for a day in test tubes

TABLE 8

*Adsorption of methylene blue and alizarine red R from 0.1 per cent solutions by samples from podzols and brown forest soils*

Milligrams per gram soil

PROFILE NUMBER	SOIL TYPE	HORIZON	AMOUNTS ADSORBED		PROFILE NUMBER	SOIL TYPE	HORIZON	AMOUNTS ADSORBED	
			Methyl- ene blue	Aliza- rine red R				Methyl- ene blue	Aliza- rine red R
1	Brown forest soil (typi- cal)	A	10.85*	2.73	2	Podzol	A <sub>2</sub>	3.56	0.00
		B <sub>1</sub>	4.55	1.00			B <sub>1</sub>	1.74	0.69
		B <sub>2</sub>	3.93	0.73			B <sub>2</sub>	1.05	1.08
		C <sub>1</sub>	1.14	0.66			C <sub>1</sub>	0.74	1.00
		C <sub>2</sub>	1.59	0.54			C <sub>2</sub>	0.43	0.00
3	Brown forest soil (accli- matic)	A	62.18†	4.75	4	Iron podzol	A <sub>2</sub>	2.76	0.00
		B <sub>1</sub>	4.50	0.00			B <sub>1</sub>	1.67	0.60
		B <sub>2</sub>	2.45	0.20			B <sub>2</sub>	1.46	0.05
		B <sub>3</sub>	2.89	0.29			C	0.95	0.00
		C	1.88	0.05					
5	Iron podzol	A <sub>2</sub>	2.18	0.00	6	Iron podzol	A <sub>0</sub>	40.95‡	4.80
		B <sub>1</sub>	2.00	0.75			A <sub>2</sub>	3.27	0.00
		B <sub>2</sub>	0.88	0.55			B	1.67	3.26
		C <sub>1</sub>	0.67	0.24			C	0.40	0.20
		C <sub>2</sub>	0.77	0.39					
		C <sub>3</sub>	0.74	0.20					
7	Iron humus podzol	A <sub>2</sub>	3.21	0.00					
		B <sub>1</sub>	3.37	1.30					
		B <sub>2</sub>	4.36	0.84					
		C	2.78	0.00					

\* From 17 mgm. added.

† From 64 mgm. added.

‡ From 43 mgm. added.

with 10 cc. of an aqueous solution containing 1 mgm. per cubic centimeter of methylene blue or alizarine red R. The next day 5 cc. of the clear solutions was pipetted off from each test tube; the amount of dye present was colorimetrically estimated; and, from the results, the amounts adsorbed were calculated in milligrams per gram of soil (table 8). In some cases where the

amount added was totally adsorbed new amounts of more concentrated dye solution were added until the solution remained colored.

The podzol profiles show a high adsorption of methylene blue in the  $A_2$  horizon and a gradual decrease through B and C. Sometimes the adsorption in  $A_2$  is twice as high as in B, though the amount of colloids is generally low in  $A_2$ . Thus the qualitative properties of the colloids are clearly expressed. The lack of agreement with, for instance, the combining capacities reported in table 6 (5) is accounted for by the fact that the methylene blue adsorption took place at a *relatively* lower pH, i.e., nearer to the isoelectric point, in the case of the B and C samples. The more strongly acidoid  $A_2$  shows, therefore, a relatively greater adsorption of the dye than that corresponding to the combining capacities at pH 7. Only in the case of the iron humus podzol do we find a higher adsorption in B than in  $A_2$ . As has been stated, however, this type of podzol is characterized by a very high content of colloids in the B horizon; furthermore, this horizon is rich in organic matter. These things account for the difference. The adsorption of alizarine red gives, as predicted, even more distinct proofs of the qualitative properties of the different soil horizons and their colloids. Being an acid dye it is not adsorbed at all in the  $A_2$  horizon just as it is not adsorbed by the pure silica. In the B horizon with its comparatively high content of ampholytoid material there is always a very marked adsorption. In the deeper parts of the profile there is a decrease, as might be expected, because of the decline in the amounts of colloids present. Sometimes no adsorption was found in C, which would perhaps seem remarkable. It should be remembered, however, that the soils investigated are "young" soils developed during the few thousand years since the last glaciation. The parent material, a moraine, consists chiefly of mechanically disintegrated rocks. These rocks, porphyries, sandstones, and granites, are composed of minerals which perhaps do not adsorb alizarine red R, just as they, according to Hardy, do not adsorb alizarine S (1, p. 155). Thus the method employed, in cases like those here presented, would possibly give a fairly accurate determination of the depth to which the soil forming processes have proceeded. In the  $A_0$  layer the adsorption of methylene blue is very high, as indicated by the results in profile 6. This might also be expected, because of the high content of acid humus. It is noteworthy, however, that the alizarine adsorption also is high in  $A_0$ . This must certainly depend on basoid or ampholytoid organic compounds in the soil colloid complex, such as the proteins, since the mineral compounds, judged from the properties of the  $A_2$  horizon, hardly can adsorb this dye in appreciable amounts.

In the case of the brown forest soil we find the same type of methylene blue adsorption as in the podzols, i.e., the adsorption is very high in A and decreases gradually through B and C. This should indicate that the acidoid character of the soil colloid complex decreases with increasing depth. But it must be remembered that the amount of colloids, especially of the organic matter, is higher in the upper parts of the profile, and thus the results are not conclusive.

The alizarine adsorption varies in exactly the same way: it is high in A and then decreases. This does not necessarily mean, because of the difference in the amounts of colloid present, that the basic residues in the colloids decrease with increasing depth. But the results show a clear difference between the podzols and the brown forest soils. In the case of the latter soil type ampholytoid compounds of the colloid complex remain in appreciable amounts throughout the entire A horizon, as well as in B, as against the total absence of basic residues strong enough to adsorb alizarine in the A<sub>2</sub> horizon of podzols. The conclusion must be that the type of weathering leading to the formation of a brown forest soil is of far less acid character than that leading to a podzol.

The case of the aclimatic brown forest soil is interesting. At first we note the very high adsorption of methylene blue in A, even higher than the one recorded for A<sub>0</sub> of the iron podzol 6, though the percentage of organic matter as well as the acidity is lower. But nearly all organic matter is probably colloidal in the case of the brown forest soil mull as against the coarse nearly undecomposed organic residues of the podzol duff, and this certainly accounts for the difference. The adsorption of methylene blue then decreases through B and C in about the way described for the other soils. The alizarine adsorption is most interesting. It is high in A, absent in the upper B, increasing in the deeper parts of B, and very low in C. This means that the ampholytoids present in B<sub>1</sub> have basic residues so weak as not to be able to adsorb any alizarine anions, because of acidoids present, though the amounts of ampholytoids are rather high [compare the high amounts of sesquioxides found with the acid-oxalate method (4, p. 149)]. The results show that the aclimatic brown forest soil, in its properties, stands between the typical brown forest soil and the podzols. The type of weathering is less acid than that of the podzols, allowing sesquioxides to remain as an important part of the colloid complex even in the upper parts of the profile. At the same time, however, the acidoids are strong enough to "neutralize" the basoid groups of the ampholytoids to a far greater extent than in the typical brown forest soils.

The results of experiments with dye adsorption may be summarized as follows. Basic dyes, such as methylene blue, are strongly adsorbed by soil colloids rich in acid residues. Acid dyes, such as alizarine red R, are in the same manner adsorbed by soil colloids rich in basic residues. The qualitative and approximately quantitative properties of the colloids thus established aid in the study of the soil profiles. They also permit a comparison between different soil types. The theory of isoelectric weathering is confirmed by the results obtained, and it could be shown that the brown forest soils are the result of a less acid weathering than are the podzols. It could even be demonstrated that an aclimatic brown forest soil in its colloidal properties takes a middle position between podzols and typical brown forest soils. The alizarine adsorption may also perhaps under certain conditions be used as a means of determining how deep the soil forming processes are apparent in a soil profile. Whether the dyes here used are the most suitable may be left to further inves-

tigations. Hardy has proposed the use of haematoxylin (1, p. 164). Many other dyes, both basic and acid, should be tested in order to determine the best method.

#### SUMMARY

The podzol and brown forest soil profiles dealt with in the first and second parts of this paper are further investigated as to their power of dye adsorption.

Experiments with artificial aluminosilicates show that the dye adsorption is comparable to the ion adsorption of colloids.

The adsorption of acid and basic dyes is a promising method for a rapid and conclusive investigation of the colloidal properties of a soil. For a tentative investigation the colorimetric method described in this paper seems to give enough information. Scientifically more conclusive results will be obtained by determining the adsorption of the dye at a definite pH value which should lie above and below the isoelectric point for the basic and acid dye respectively.

Methods such as those used in the three parts of this paper will permit a rapid and conclusive comparative study of the soil forming processes in different soil regions. Acid-oxalate determinations of the reactive part of the colloids or the easy methods of investigating the amphoteric properties of the soil colloids—especially determinations of the ultimate pH, the pH of exchange neutrality, the combining capacity at different pH values, and the adsorption of acid and basic dyes—are very valuable in comparative studies where many analyses are necessary. For a thorough knowledge of the soils total chemical analyses are necessary; for many purposes, however, they may be confined to a few type specimens of the soils and the comparative study made with the easy and rapid methods here applied.

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## BOOK REVIEW

*Humus. Origin, Chemical Composition, and Importance in Nature.* By SELMAN A. WAKSMAN, professor of soil microbiology, Rutgers University, and microbiologist, New Jersey Agricultural Experiment Station. The Williams & Wilkins Co., Baltimore, 1936. Pp. xi + 494, figs. 45, tables 63. Price \$6.50.

One who has followed the numerous reports of the author's researches on the microbial transformation of organic materials is familiar with the subjects receiving particular emphasis in this monograph, for it is about the subject matter of these reports that the treatise is developed and considerably extended. It is far more than a recapitulation of this material, involving a thorough and careful consideration of the extensive literature on the properties and importance of the organic formations found in nature. The author's investigations of the last fifteen years have yielded much new information which has revised the conception of the nature of soil organic matter and of the processes leading to its formation. His critical and keen interpretation of the facts, his originality of approach, and his broad appreciation of the many aspects of the subject have resulted in the creation of logical order from the confused and seemingly contradictory evidence in the scientific literature. In any attempt to coördinate such conflicting views as pervade the subject it is almost inevitable that there will be divergence of opinion. It is with temerity that most persons would assume the task; particular commendation is therefore due the author for having so effectively surmounted the difficulties.

According to the author: "This book is an attempt to tell the story of humus, its origin from plant and animal residues, its chemical composition, its physical properties, its importance in nature, especially in soil processes and in plant growth, and finally its decomposition. . . . Humus is a complex aggregate of brown to dark colored amorphous substances, which have originated during the decomposition of plant and animal residues by microorganisms, under aerobic and anaerobic conditions, usually in soils, composts, peat bogs, and water basins. . . . The chemical composition of humus is determined by the nature of the residues from which it is formed, by the conditions of its decomposition, and by the extent to which it is decomposed. Chemically, humus consists of numerous organic complexes, the major group of which consists of lignins and lignin derivatives and of proteins; a minor group contains carbohydrates, fats, organic acids, alcohols, and other carbon compounds."

The use of such terms as "humins," "ulmins," "crenic acid," "apocrenic acid," and others as they are applied to preparations is considered by the

author to be unwarranted. He prefers to characterize humus by the relative proportions of the various chemical substances such as cellulose, hemicelluloses, lignin, proteins, etc., which it contains. More information concerning lignin in particular, its composition, characteristic properties, and decomposition, is still needed to complete an understanding of the behavior of the soil organic matter.

Although there is considerable emphasis upon the chemical and physical properties of humus as a whole and of its specific constituents, principal attention is directed to the activities of microorganisms in determining the nature of the residues and products of decomposition which characterize the humus. Among the organic substances considered, other than soil humus, are plant residues, animal manures, peat, coal, and organic matter in waters. The practical importance of these materials is indicated, but chief attention is focussed upon the fundamental principles which should be the basis of agricultural practices.

A list of chapter titles inadequately suggests the variety of subjects considered: The rôle of humus in the organic cycle in nature; Nature and characteristics of humus; The rôle of humus in plant nutrition; The changing conceptions concerning the chemical nature of "humus" and "humic acids"; "Humification" of organic matter in soils and in composts, and methods of "humus" determination; Origin of humus; Isolation of definite organic chemical compounds from humus; Chemical nature of humus as a whole; Humus formation in composts, animal manures, and green manures; Humus in forest and heath soils; Humus in mineral soils (field, grassland, garden, and orchard); Humus in peat and in coal; Organic matter formations in water systems; Physical and physicochemical properties of humus; Decomposition of humus in nature; Presence in humus of specific substances which have an injurious or a beneficial effect upon growth of plants, animals and microorganisms; Utilization of various forms of humus for agricultural and industrial purposes; Soil humus and the science of pedology; Humus as an organic system; Appendix—Methods of analysis of humus and of certain humus constituents.

There are 1311 items in the bibliography, including many more than this number of individual references. There are both author and subject indexes.

This volume should prove useful to all who are concerned with the most profitable use and conservation of the natural resources of organic materials, particularly to students of soils and plants.

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## INVESTIGATIONS OF RED SOILS OF ATTICA, GREECE

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The soils of the region adjacent to the Mediterranean recently have become the subject of systematic studies. The interesting works of Del Villar (4), Blanck (2) and his collaborators, Reifenberg (8, 28) Harassowitz (14, 15) and a number of other eminent investigators are proof of the importance of such studies. Practically all these authors have the same point of view as that advanced by Neumayer (23), namely, that the typical Mediterranean red soils ("terra rossa") are formed on limestone. Fuchs (9), about the same time, supported this view more definitely, asserting that typical red soils ("terra rossa") are laid only on pure and hard white limestone and never on soft marl or chalk-like ones.

It is universally admitted that typical red soils are always found on a lime parent rock. Zvorykin (39) states that under the climatic conditions of Middle Europe, in Moravian Karst, where Devonian limestone is very common, we have red clay which serves as parent rock for the development of podzolic soils, which are formed by the washing out of  $\text{CaCO}_3$  and the replacement of exchangeable bases from the parent rock. Jahn (16) in his geological sketch calls this type of red clay "terra rossa."

Under Greek conditions, at moderate altitudes, soft limestones generally do not develop typical red soils, but we have soils of the rendzina type varying in color from gray to dark gray. In this respect our results agree with those of Fuchs (9) and Glinka.

On the hillsides of Thessalia, however, in the vicinity of the city of Larissa, the rendzina type of soils under the influence of sheet erosion formed reddish brown soils with granular structure. These soils are more thoroughly saturated with the bivalent cations Ca and Mg than are the typical red soils. In this region we find a decidedly complicated type of soils, which are influenced by the topographic conditions. We encounter surfaces that are entirely washed off from the parent rock, which thus remains exposed; surfaces occupied by rendzina soils; and, lastly, surfaces with strips of reddish brown soils.

We are of the opinion that, under the conditions mentioned, a partial application of the theory of Stremme (36) as to the origin of red or reddish brown soils under the influence of erosion can be made. Robinson (29) is quite right, however, in stating that Stremme's theory cannot be applied to the entire Mediterranean region.



Although it is well known that red soils are formed on rocks that contain large quantities of  $\text{CaCO}_3$ , we have observed in our studies of soil profiles from Thessalia, near the village of Tsagezi, on riparian terraces composed of weathered carbonate-free schists, a pale reddish tint on the main ocher ground. On the upper horizon we noticed very distinct red spots as well as narrow red veins, the latter being spread over all the profile to a depth of 105 cm. Lower horizons than these could not be obtained with ordinary tools.

During the last four years, while visiting practically all of Thessalia, Macedonia, and Attica, we made field observations and collected many monoliths for laboratory study. On the basis of these observations and laboratory investigations, the latter not being quite completed, we propose that reddish soil formations are found throughout these regions, the differences within the soils varying with the different parent rocks and with the topography.

In 1924, while working as an investigator at the Pedological Laboratory of Brünn, the junior author made several excursions in Moravia with Professor Afanasiev, who drew attention to the presence in the author's monoliths of pale reddish hues in horizon  $A_2$  of certain podzolic soils which were developed on carbonate-free parent rocks. On these grounds Afanasiev advanced a theory that the red soil formation throughout all Middle Europe is just beginning. In any event this question deserves a wider and more systematic study.

In some regions, in Thessalia and especially in Macedonia, the red soils are subjected to the saline process and, under the influence of more or less advanced saturation with Na, are gradually changing their morphology, becoming darker in color, especially in the B horizons, and are forming hardpan. For the most part, however, it is possible to recognize that we have before us a transformed red soil. Glinka (12, p. 414), who has observed red soils in the neighborhood of Salamanka, Spain, also speaks of the existence of saline and non-saline red soils. Del Villar (5) states, too, that in red soils there is a considerable quantity of absorbed Na. In Macedonia the influence of Na is very pronounced, especially in carbonate-free red soils. Having already undergone a degradation process, the saline red soils of Greece contain, for the most part, no adsorbed Na. In the laboratory one can discover only traces of the influence of this cation.

This phenomenon could be explained, according to investigations of Zvorykin and Katakosinos<sup>1</sup> and of Zvorykin (40), by the lowering of the base level of erosion, which is closely connected with the lowering of the ground water level. This question is thoroughly treated by Polynov (27) in his investigation of the Don Valley, Russia.

The greatest transformations of the soils of Greece, particularly of Attica, are due to the influence of sheet and gully erosion. Periodical winter floods, caused by the very uneven distribution of rainy seasons and by the topography,

<sup>1</sup> Zvorykin, I., and Katakosinos. *Considerations générales sur la salinité des terres dans la plaine de Salonique*. Unpublished.

destroy the soil and transport it to lower spots. In this manner the main type of a new soil originates.

The action of erosion must be attributed to the fact that in regions of typical red soils we very often find yellow and even gray soils. In studying the morphology of the profiles, not taking into account the physico-geographical conditions, we can find inside the boundaries of small regions a mixture of soil types belonging to different zones, as is shown by the map of Stremme (37, p. 18). In the vicinity of Salonica, in Macedonia, the same author shows brown and forest soils, instead of white and black alkali soils, and degraded black alkali soils formed on red soils.

On the basis of the study of the action of erosion, red soils may be divided into two groups as follows: first, soils formed *in situ* on a stable topography, and secondly, transported soils deposited on a topography still in the process of formation. The latter are sometimes very deep and consist of buried horizons of red soils alternating with stony deposits, the pebbles of which may be smooth or rough according to the character of their origin, alluvial, deluvial, or proluvial,<sup>2</sup> as explained by Pavlov (25, 26). Similar erosion processes undoubtedly change the mechanical and the chemical composition of the red soils, as discussed by Agafonov (1) in his investigation of the red soil of Brazil.

On the basis of the foregoing facts, in order to form an idea of the red soils of Greece it seems necessary first to study soils located in a stable topographic position, where the process of new formation has had time to manifest itself on the maturity of the soil profile. Only under such conditions can we speak of the age of the soil, because, as Sokolov (33) points out, the age of the soil does not depend directly on the age of the parent rock, but rather on the final stages of the orographic elements, at which point the geological processes are substituted by soil formation.

Having on hand these experimental data, we chose Attica as a typical province for the study of red soils.

To characterize Attica, from the climatic point of view, we have used the records for the last 50 years of the Meteorological Observatory of Athens, such as temperature, quantity of rainfall, and relative moisture, published by Eginitis (6), and also the evaporation records of Findiklis (7) for the period 1894-1928. The evaporation records were obtained by means of a Pich evaporimeter. Findiklis remarks that these figures may be somewhat high, but for our work such observations are of marked interest even though they are only of a comparative nature. The results of these observations are shown in table 1.

From the table it is evident that the rainfall for the entire year is divided into two periods: the wet winter period and the dry summer one. With the wet period correspond the lowest temperature of the air and the highest relative

<sup>2</sup> The terms "deluvial" and "proluvial" were introduced by Pavlov, the latter to designate the unsifted deposits formed at the estuaries of mountain valleys. These deposits are products of sudden floods.

TABLE 1  
*Climatic records for Attica, 1853-1928*

	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL OR AVERAGE
Rainfall, 1858-1928*	52.14	39.37	36.10	19.51	20.91	13.75	6.34	8.66	13.38	41.25	71.84	66.64	392.83
Temperature, 1853-1903†	8.77	9.58	12.21	15.85	20.78	25.17	28.06	27.63	24.17	19.80	14.42	10.69	18.14
Relative moisture, 1860-1903	72.4	71.1	67.1	62.7	58.2	53.6	46.0	45.5	53.8	65.2	72.1	73.7	61.4
Evaporation, 1894-1928‡	65.81	67.39	90.97	116.94	155.13	196.48	251.02	235.55	178.39	112.86	78.40	64.69	1,613.73

\* After Eginitis (6) and Findiklis (7).

† After Eginitis (6).

‡ After Findiklis (7).

TABLE 2  
*Chemical analysis of soil profiles 3 and 4*

ATTICA	HORIZONS	H <sub>2</sub> O AT 105°C.	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	MnO	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	CO <sub>2</sub>	LOSS OF ORGANIC MATTER ON IGNITION	TOTAL
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Profile of Agios Andreas	0-10	2.19	41.85	8.18	5.24	0.31	20.38	1.23	1.20	0.93	0.15	0.01	14.49	5.92	99.89
	10-32	1.75	23.92	6.95	3.04	0.26	34.32	0.89	0.87	0.68	0.26	0.01	25.67	3.03	99.90
	32-60	1.30	24.49	6.89	2.99	0.09	35.39	0.81	0.89	0.63	0.11	trace	25.19	2.47	99.95
	60-120	2.77	28.37	7.78	4.54	0.07	31.62	0.89	0.94	0.68	0.16	0.02	22.35	2.50	99.92
Profile of Halandri	0-27	1.84	59.40	10.84	5.62	0.15	10.02	1.21	1.31	0.87	0.12	0.03	6.15	4.47	100.09
	27-43	2.12	60.98	11.73	5.80	0.15	7.82	0.79	0.97	1.16	0.16	0.02	5.27	5.08	99.93
	43-55	2.36	52.89	11.62	5.16	0.18	13.82	0.76	1.35	0.48	0.19	trace	8.91	4.53	99.89
	55-85	1.83	51.54	9.81	4.65	0.13	16.83	1.22	1.09	0.79	0.19	trace	10.95	3.12	100.32
	85-120	1.16	48.10	8.64	3.90	0.14	20.09	0.95	1.21	0.46	0.19	trace	13.17	3.19	99.94

moisture. The average temperature in the coolest month of January is above 0°C.

Kappen (17), on the basis of meteorological records, classifies the climate of Attica as warm with a dry summer. De Martonne (21) characterizes the climate of all Greece as Mediterranean-Continental with a mild and wet winter period followed by a dry summer, during which the conditions become similar to those of a desert.

Findiklis (7), comparing the evaporation records of Athens with those obtained in a near-by desert, such as Turkestan, Sudan, or Egypt, concludes that they are very similar. If the classification of MacDougal (20) is followed, the climate of Attica during the dry period and at a moderate altitude can be classified as that of a desert. Such conclusions seem to be more justified as the average annual rainfall for Greece, as a rule, is not great and, furthermore, during the summer months it drops to a minimum.

We are of the opinion that the occurrence of two sharply defined periods of rainfall is of great significance for the various soil forming processes; for example, during the rainy period the soils become saturated to a considerable depth and remain in this state from November until May, when the soil begins to dry.

It seems possible that the soils of Greece undergo, during the year, two periodical influences; namely, first, the eluviation, during the wet period, at which time the soil solution descends; and secondly, the illuviation, during the dry period, at which time the soil solution ascends. The presence of colloids complicates the situation. If the colloids are reversible, as in the case of soils saturated with Na, then under Greek conditions in the absence of periodic or continuous uplifting of the ground water, the formation of solodi from the black alkali soils follows.<sup>3</sup> Under such conditions we have a sharp differentiation of the profile into horizons, alteration of the structure, and a speedy decomposition of the colloidal part of the soil, especially in the upper horizon.

Reifenberg (8, 28) was the first to study soils that contain irreversible and mutually coagulating colloids. We are of the opinion that the red soils of the Mediterranean region are very appropriate material for such study.

#### STUDY OF SOIL PROFILES

We know very definitely that color and the conditions of the deposition of "terra rossa" are constant at different altitudes, as stated by Robinson (29) and by Zvorykin (39), but the soils formed on it have different characters depending on the physico-geographic characteristics of the country, which in turn determine the maturity of the soil profile. This is substantiated by chemical analyses.

From our monolith collection, we selected profiles 3 and 4 for this study.

Monolith 3 (plate 1, fig. 1) was taken on December 14, 1935, along the main road from Athens to Marathon, in Attica, 2 km. south of the chapel of

<sup>3</sup> See footnote 1.

Agios Andreas on the ridge of a terrace elevated 40–45 m. above sea level and abruptly sloping down to a beach. The ridge of the terrace under the influence of gully erosion is cut by a deep ravine having steep slopes. The terrace is composed of limestone rocks weathering sometimes to a considerable depth. Groves of pine trees (*Pinus halepensis*) alternate with strips of ploughed fields. The mountains overlooking the terrace are composed of hard white and grayish white limestones.

### *Description of profile 3*

Horizon I, 0–10 cm. The surface is covered with a very thin (0.5 cm.) broken layer of decomposing pine needles. The horizon is moist; light red with a yellowish tint and with a gray stain of humus; structureless, having in the lower part small quantities of lime concretions of ovoid form; very loose (plate 2, fig. 1); and fragile. This horizon is very rich in  $\text{CaCO}_3$ .

Horizon II, 10–32 cm. This horizon is moist, pale reddish, soft, loose, and structureless. It contains a large quantity of soft ovoid concretions with a yellowish bloom and also some lumps of limestone (plate 2, fig. 2), roughened by the process of weathering and representing the remnants of the original parent material. The hardened nuclei of these remnants are covered with tuff. This horizon is very rich in  $\text{CaCO}_3$ .

Horizon III, 32–60 cm. This horizon is slightly moist, more compact than the former and lighter, and the reddish straw colored soil is pierced with limy veins and concretions as described for horizon II. Often we encounter rough remnants of hard limestone. This horizon is very rich in  $\text{CaCO}_3$ .

Horizon IV, 60–120 cm. This horizon is slightly moist and very hard with its reddish background permeated by lime veins, bands, and spots. A large number of concretions and remnants of tuff-covered lime are encountered. It is very rich in  $\text{CaCO}_3$ .

The process of soil formation and the character of the topography of the land disclose a prolonged and deep weathering of the limestone deposits, which have been converted for a considerable depth into a loose mass, containing secondary products of weathering in the form of lime concretions, leaving behind only rough pieces of parent rock with a hard crust on one side. Profile 3 can be taken as an example of the process of red soil formation under forest conditions.

Monolith 4 (plate 1, fig. 2) was taken on December 15, 1935, near the village of Halandri, Attica, at a height of 215 m. above sea level on a flat and broad ridge of a terrace rising above the bank of the river Kifissos.

The ridge of the terrace is cut through by a gully erosion, which forms crevices with vertical sides occupying a limited portion of the ridge of the terrace. The inclination of the land, in sheet erosion, is comparatively slight, and the erosion cannot cause great change in the character of the soil.

Besides erosion, another factor contributes to the alteration of the soil hori-

zon. For many years these soils have been planted to vegetable crops and vineyards. As a result, the ground has been tilled very deep; therefore, we may consider the first two horizons (the second at least in its upper part) as thoroughly mixed and consequently as not having maintained their original material, which is the case for the most of the arable soils of Attica.

It is interesting to note that under the conditions of Attica fruit trees as well as olives at a certain age suffer from drought; often the tops (foliage and branches) of trees are dried up. This phenomenon might be explained on the basis of the physical structure of the lower horizons of the soil as described in the following section.

#### *Description of profile 4*

Horizon I, 0-27 cm. The surface is dark red, covered with a thin (0.5 cm.) blanket of turf, is structureless, becomes loose when dried up, contains a good many roots, and is very rich in  $\text{CaCO}_3$ .

Horizon II, 27-43 cm. This horizon is moist, dark red, slightly brighter than the foregoing, and has preserved the fine granular structure especially in the lower portion, notwithstanding cultivation. It is permeated with fine thin roots. In the dry state it is loose and dusty. It also contains large quantities of  $\text{CaCO}_3$ .

Horizon III, 43-55 cm. This horizon is moist and dark red but somewhat lighter than the preceding and much more compact, with thin lime veins resembling micellium and with a few white lime spots. In it we encounter scanty wilting roots and insect borings. This horizon is also rich in  $\text{CaCO}_3$  content.

Horizon IV, 55-85 cm. This horizon is moist, brick-red colored with a pale ocher tint and lighter than the preceding, extremely compact, permeated by thin vertical flaws, and somewhat porous. It has white spots and small lime concretions as thin veins. It contains a small quantity of decaying roots and much  $\text{CaCO}_3$ .

Horizon V, 85-120 cm. This horizon is slightly moist, light reddish with a straw tint, entirely pierced by thin veins of lime, porous, extremely hard and compact even in its slightly moist state. It frequently occurs in the form of a lime hardpan. Occasional small dried out roots enriched with  $\text{CaCO}_3$  are found.

The soil described has a mature profile where the processes of soil formation are very pronounced. Accumulations of lime occur in the compact low horizons. This compactness attains its maximum in the lower horizon, which becomes impervious to the root system. This accounts, we believe, for the drying of the tops of the fruit trees, a phenomenon analogous to that which goes on in solonetz or solonetz-like soils. The hardened horizon affects the roots in the manner described, notwithstanding the fact that it contains neither  $\text{Na}_2\text{CO}_3$  nor sodium. This soil is very typical of the wide and open valleys of Attica.

*Interpretation of chemical analyses*

In studying the chemical properties of the respective horizons of the profiles total analyses were made, the exchangeable bases were determined by the Shmuk (30) method, and the Gedroiz (11, p. 282-287) 5 per cent KOH extraction method was applied. We also deemed necessary the determination of the hydrogen-ion concentration (pH), which we carried out electrometrically. The Gedroiz method was used for the determination of the degree of decomposition of the colloidal part of the soil. In our case we have considered indispensable the application of this method of Gedroiz because of the presence of hardpan in profile 4. Such hardpans are commonly formed on black alkali soils. In this particular case the tops of the fruit trees were dried up, which led us to believe that the soils under investigation were probably saturated with sodium.

TABLE 3

*Chemical analyses of soil profiles 3 and 4 calculated on mineral content minus humus and CO<sub>2</sub> content*

ATTICA	HORI- ZONS	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	MnO	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>
	cm.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Profile of Agios Andreas	0-10	52.65	10.28	6.57	0.39	25.04	1.53	1.50	1.17	0.19	0.01
	10-32	33.59	9.75	4.27	0.36	48.20	1.25	1.21	0.95	0.36	0.01
	32-60	34.09	9.51	4.05	0.12	48.93	1.11	1.22	0.86	0.15	trace
	60-120	29.75	10.35	5.76	0.09	42.07	1.18	1.24	0.89	0.21	0.02
Profile of Halandri	0-27	66.46	12.13	6.28	0.16	12.39	1.34	1.46	0.96	0.13	0.03
	27-43	68.01	13.07	6.46	0.16	8.75	0.87	1.08	1.29	0.18	0.02
	43-55	61.09	13.42	5.94	0.21	15.96	0.87	1.56	0.55	0.22	trace
	55-85	59.98	11.42	5.04	0.14	19.58	1.41	1.26	0.91	0.28	trace
	85-120	56.84	10.21	4.61	0.16	23.20	1.12	1.43	0.53	0.21	trace

The results of our chemical investigations are shown in tables 2 and 3. The results in table 3 are calculated on mineral substances of the soil, minus the humus and CO<sub>2</sub> content. These tables indicate that the organic matter of the soils under investigation diminishes with the increase in depth and that this decrease is more pronounced in profile 3 (Agios Andreas) than in profile 4. The largest quantities of SiO<sub>2</sub> are found in the first horizon of profile 3 and in the first two horizons of profile 4. The SiO<sub>2</sub> decreases in the lower parts of the profile, particularly in profile 3.

The R<sub>2</sub>O<sub>3</sub> accumulates in the surface horizons, the Fe<sub>2</sub>O<sub>3</sub> more so than the Al<sub>2</sub>O<sub>3</sub>.

On the other hand the CaCO<sub>3</sub> in general increases with depth. It is interesting to note that the quantity of CaCO<sub>3</sub>, as a general rule, is very large in the horizons of profile 3 and is greater even than that of SiO<sub>2</sub>, notwithstanding the fact that the secondary lime formations, have, in the preparation of the sample, been sifted out by a 2-mm. sieve.

MgO is fairly evenly distributed among the horizons of profile 3, but in profile 4 it decreases somewhat in horizons II and III and again increases in horizons IV and V.

It is interesting to note that the  $K_2O$  content is, for the most part, greater than that of  $Na_2O$ .

The  $P_2O_5$  content in the upper horizons is often poorer than in the lower ones. This can be seen very clearly in profile 4. In profile 3, sharp differences in the  $P_2O_5$  content are apparent in horizons I and II. These figures, of course, represent the entire quantity of  $P_2O_5$  found in the horizons and not that which is assimilated by the plants.

Zvorykin (39) conducted investigations in the Moravian Karst on soils formed from red loam overlying hard Devonian limestone. These soils developed zonal characteristics—podzolic and degraded rendzina—under the influence of local physico-geographic conditions. The results of these investigations can be compared with those obtained in Attica by the writers.

The podzolized soils of the Moravian Karst infrequently retain the reddish tinge in horizon  $A_2$ , notwithstanding the leaching out of  $R_2O_3$  from the surface layers of the profile. Horizon B resembles "terra rossa" in its color but is distinguished from it by its nutty structure (plate 1, fig. 3). The B horizon of slightly degraded rendzina is still more similar to the red soil formations observed in Attica.

The reddish clay of the Moravian Karst (horizon B) is far richer in  $Al_2O_3$  but much poorer in  $CaCO_3$  than are the profiles of Attica that were studied. Similar results, based on total analyses, are found in horizon B of slightly degraded rendzina soils of the Moravian Karst region (table 4).

From a comparative study of the soils of Attica and of the Moravian Karst we observe that the composition of the red soils varies considerably with the physico-geographical condition, notwithstanding the underlying limestone parent material. The data of Reifenberg corroborate this conclusion.

The results of base-exchange determinations on the soils of Attica (table 5) reveal the following phenomena: notwithstanding the large quantities of limestone, as shown by the total analyses, the amount of absorbed Ca is relatively small; absorbed Mg is also found in small quantities; and the Na content is too small to impart to the soil solonetzic properties.

The reaction of the profiles of Attica, expressed in pH, is neutral or slightly alkaline.

From the data in tables 6 and 7 we can compare the exchangeable base content of the soils of Attica with those of the Moravian Karst and Thessalia. The soils of Thessalia, which are reddish brown, rest on soft limestone. In this manner we can compare, on the one hand, the properties of soils found under different climatic conditions, such as the soils of Attica and the Moravian Karst, and, on the other hand, the soils of Attica and Thessalia found under nearly identical climatic conditions, the difference being of a petrographical nature.

From tables 6 and 7 it is apparent that the podzolic horizons of the Moravian Karst profile, as would be expected, are unsaturated with bases and have



a low pH, but the underlying horizon B is of an entirely different character. In this case the quantity of bases rises sharply, and judging from the pH of the soil it is fully saturated with bases. Horizon B, 43-79 cm. a very slightly degraded rendzina soil, is practically saturated with bases, and has a higher index of pH.

In this manner under the same physico-geographic and petrographic condition and environment of the soils, we observe the same degree of saturation with bases, regardless of the different losses of organic matter on ignition.

TABLE 4  
*SiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> extracted with 5 per cent KOH from Moravian Karst soils*

MORAVIAN KARST	HORI- ZONS	DEPTH	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	CaO	MgO	CO <sub>2</sub>
		cm.	per cent	per cent	per cent	per cent	per cent	per cent
Podzolic soil (degraded rendzina)	A <sub>1</sub>	3-8	76.28	12.74	3.83	0.64	0.67	....
	A <sub>2</sub>	8-23	78.93	13.12	4.74	0.66	0.88	....
"Terra rossa"	B	23-52	62.73	22.59	5.52	1.67	1.51	0.11
"Terra rossa" of slightly degraded rendzina.....	B	43-79	63.66	18.29	8.06	2.06	1.39	0.26

TABLE 5  
*Exchangeable bases of Attica soil profiles*

ATTICA	HORI- ZONS	Ca	Mg	Na	Ca	Mg	Na	TOTAL EX- CHANGE- ABLE BASES	pH
	c. m.	per cent	per cent	per cent	m.e.	m.e.	m.e.	m.e.	
Profile of Agios Andreas	0-10	0.669	0.017	0.007	33.45	1.41	0.32	35.18	7.38
	10-32	0.578	0.014	0.008	28.90	1.16	0.36	30.42	7.53
	32-60	0.396	0.022	0.005	19.80	1.83	0.22	21.85	7.53
	60-120	0.426	0.017	0.012	21.30	1.41	0.52	23.23	7.60
Profile of Halandri	0-27	0.481	0.024	0.004	24.05	2.00	0.17	26.22	7.57
	27-43	0.449	0.058	0.003	22.40	4.83	0.13	27.36	7.53
	43-55	0.327	0.010	0.003	16.35	0.83	0.13	17.31	7.42
	55-85	0.448	0.048	0.008	22.40	4.00	0.36	26.76	7.20
	85-120	0.213	0.034	0.010	10.65	2.83	0.43	13.91	7.16

The base saturation of the red horizons of the Moravian Karst soils exceeds considerably that of the soils of Attica, notwithstanding the fact that the latter are enormously rich in CaCO<sub>3</sub> and contain a considerable quantity of organic matter. The degree of saturation of the soils from Thessalia is also considerably higher than is that of the soils from Attica, which prove to be the poorest of all the soils so far studied by us.

We are of the opinion that the degree of saturation depends not only on the quantity of organic substances, clay, and CaCO<sub>3</sub> present, as stated by Mattson

(22) and Williams,<sup>4</sup> but also on the physico-geographic conditions, especially of the "soil climate," as it is termed by Novak (24), and on the degree of solubility of the limestone upon which the soil rests.

One may assume that the degree of solubility is higher for the soft limestones than for compact and hard ones. Sigmond (31) supports this opinion in his study of the application of the methods of Hissink for the exchangeable bases of soils containing large quantities of  $\text{CaCO}_3$ . It is possible, therefore, that the presence in Greece of rendzina found on soft limestone may be explained by the high solubility of the latter. For this reason the rendzina, but not the red soil, has a high content of absorbed calcium.

TABLE 6  
*Exchangeable cases of Moravian Karst soils*

MORAVIAN KARST	HORIZONS	DEPTH	Ca	Mg	Ca	Mg	TOTAL Ca AND Mg	pH	LOSS OF ORGANIC MATTER ON IGNITION
		<i>cm.</i>	<i>per cent</i>	<i>per cent</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>		<i>per cent</i>
Podzolic soil (degraded rendzina) "Terra rossa" "Terra rossa" of slightly degraded rendzina	A <sub>1</sub>	3-8	0.135	0.004	6.75	0.33	7.08	5.00	8.65
	A <sub>2</sub>	8-23	0.153	0.007	7.65	0.05	7.70	5.05	4.92
	B	23-52	0.843	0.001	42.15	0.08	42.23	6.90	11.04
	B	43-79	0.813	0.009	40.65	0.73	41.48	7.58	6.95

TABLE 7  
*Exchangeable bases of Thessalia soil profile*

THESSALIA	HORIZONS	DEPTH	Ca	Mg	Na	K	Ca	Mg	Na	K	TOTAL EXCHANGEABLE BASES	pH
		<i>cm.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	
Profile of Larissa	I	0-12	0.930	0.026	0.024	0.158	46.53	2.18	1.04	4.07	53.82	7.70
	II	12-35	0.949	0.202	0.028	0.183	47.49	16.71	1.21	4.68	70.09	7.64
	III	35-69	0.6257	0.122	0.021	0.180	32.28	11.00	0.91	4.61	48.80	7.74

The presence of organic matter is influenced by the degree of saturation of the soil with calcium, as has been shown in an earlier publication (39).

We believe that the causes of the higher or lower degree of base saturation of soils, even if they are within the limits of the same climatic zone, represent a very complicated question and depend on a series of complex conditions that must be treated separately.

Table 8 presents the determination of  $\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$  extracted with a 5 per cent solution of KOH from soils of Attica. An analysis of these figures shows that  $\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$  are present in very small amounts. In this respect

<sup>4</sup> Unpublished results, cited by Robinson (29).

the red soils under investigation are similar to black earths [see records of Gedroiz (10)].

In combining the figures of  $\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$  into a compound  $2\text{SiO}_2 \cdot \text{Al}_2\text{O}_3$  we will always have some  $\text{SiO}_2$  unaccounted for, and in some cases, also free  $\text{Al}_2\text{O}_3$ .

The results of table 8 indicate that the soils under investigation were not subjected to the influence of absorbed Na and that the colloidal part of the soil is not easily decomposed even under the influence of the processes of soil formation of Attica.

In studying the red soils of Macedonia, which contain large quantities of Na,<sup>6</sup> we have obtained larger quantities of  $\text{SiO}_2$  than of  $\text{Al}_2\text{O}_3$  even in the case of soils that contained  $\text{CaCO}_3$ .

Zvorykin (39), studying degraded rendzina and podzolic soils in Middle Europe, obtained much higher quantities of  $\text{Al}_2\text{O}_3$  than in the red soils of Attica; these results are typical for the podzolic zone.

TABLE 8  
 *$\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$  extracted with 5 per cent KOH from Attica soils*

ATTICA	HORIZONS	$\text{SiO}_2$	$\text{Al}_2\text{O}_3$	$2\text{SiO}_2 \cdot \text{Al}_2\text{O}_3$	RESIDUE	
					$\text{SiO}_2$	$\text{Al}_2\text{O}_3$
	cm.	per cent	per cent	per cent	per cent	per cent
Profile of Agios Andreas	0-11	0.266	0.217	0.471	0.011	.....
	10-32	0.325	0.266	0.578	0.010	.....
	32-60	0.279	0.186	0.401	0.059	.....
	60-120	0.434	0.275	0.534	0.089	.....
Profile of Halandri	0-27	0.348	0.240	0.522	0.065	.....
	27-43	0.437	0.347	0.755	0.020	.....
	43-55	0.523	0.474	0.967	.....	0.029
	55-85	0.473	0.509	0.872	.....	0.108
	85-120	0.465	0.348	0.757	0.055	.....

#### DISCUSSION

From a comparison of the morphology and the chemical composition of the soil profiles of Attica with those of Thessalia and Middle Europe, the following conclusions can be drawn:

The profiles of the red soils of Attica possess different degrees of maturity related mainly to the topographic conditions of deposition. Profile 3 is less mature than profile 4, as shown by its morphology. In the lower part of the first horizon of profile 3, rough remnants of limestone and secondary formations, in the shape of concretions, are present, which indicates that the process of soil formation is not yet completed.

Profile 4 has fully developed horizons, with small, rather deep concretions. At a depth of 55 cm., we find lime veins; and at 85 cm., a hardpan of limestone impenetrable to the roots is formed.

<sup>6</sup> See footnote 1.

The chemical analyses of both profiles, together with observation *in situ*, indicate an accumulation of lime in the deeper horizons.

The form and distribution of the accumulations of lime in a profile lead us to believe that these are products of the eluvial process that takes place during the wet season of the year. This process apparently is only slightly developed because of the poverty of soil moisture and because of the fact that the secondary products lie very near to the surface.

The comparative records of the Moravian Karst "terra rossa" show that under different physico-geographic conditions, eluviation processes are more marked and therefore no secondary products in the form of concretions occur; the  $\text{CaCO}_3$  is very energetically washed out. In illuvial horizons of "terra rossa" of the least degraded rendzina soil, however, we encounter large fragments of limestone, covered on the lower surface with a thin, broken, tough crust.

In the more podzolic varieties, the eluvial process is accompanied by an accumulation of  $\text{Fe}_2\text{O}_3$  and especially of  $\text{Al}_2\text{O}_3$  in the lower horizons, as is shown by total chemical analyses. By the eluvial process the red clay "terra rossa" of the Moravian Karst is substantially transformed into a podzolic type.

On the other hand, we note the comparatively small accumulation of  $\text{Al}_2\text{O}_3$  and especially of  $\text{Fe}_2\text{O}_3$  on the upper horizons of the soils of Attica. This indicates that the illuvial horizon (that enriched with  $\text{Fe}_2\text{O}_3$  and  $\text{Al}_2\text{O}_3$ ) is located near the surface of the soil and corroborates the opinion of Reifenberg (28) concerning the formation of red soils. This small accumulation of  $\text{Al}_2\text{O}_3$  and  $\text{Fe}_2\text{O}_3$ , regardless of the high evaporation can be explained, in our opinion, by the comparatively small amount of soil moisture and by the duration of the dry hot season, which results in but slight destruction of the soil colloidal particles which are protected by the absorbed calcium.

The red soils of Attica are less saturated with bases than are those of the Moravian Karst; this condition is due, not to the quantity of organic matter present, but to the dryness which retards the reactions that go on in the soil. Sokolovsky (34) shows that in soils formed under dry conditions, we find no considerable quantities of adsorbed calcium, notwithstanding the presence of large quantities of  $\text{CaCO}_3$ .

The augmented quantity of adsorbed bases has been pointed out by Gorshenin (13), who studied the phenomenon of degradation of the Siberian black soils and has explained this phenomenon on the basis of the influence of moisture and humification of black soils under forest conditions. Our investigation with soils of Attica under similar conditions corroborates the results obtained by Gorshenin.

The first horizon of profile 3 is more saturated with bases than is that of profile 4. Since we have studied a rather limited number of samples, we cannot state definitely the influence of the forest soils upon the degree of saturation with bases for the soils of Attica.

On the basis of soil extraction with 5 per cent KOH of profile 4, we concluded that the hardpan of the soil owes its origin, not to the saturation with sodium but to the accumulation of lime, such formations being typical for the soils of dry regions.

We are of the opinion that it is rather unreasonable, because of the slight saturation with bases, to compare, as Stebutt (35) does, the brown earths of Ramann with the red soils of the dry regions of the Mediterranean. In our studies of the brown earths of Middle Europe we found either podzolic soil varieties of forest soils or degraded black earths. In such cases an accumulation of  $R_2O_3$  is encountered in the lower horizons but never in the upper ones as in the red soils of Attica. This is proved by the analytical records of Stremme (38) and of Smolik (32).

The comparatively feeble saturation with bases of the brown earth is accounted for by the podzolic process which often reaches a very advanced state. In Attica the degree of saturation is very likely combined with more complicated circumstances, as has been partially described. Apart from this we must take into consideration the remark of Glinka (12, p. 414) that under the name of "brown earths" are seemingly described various soil formations; as an example, we can take the map of Stremme (37, p. 18).

In such a manner, if we agree to call "brown earth" every feeble podzolic soil tinted with brown and brownish hues then of course the red soils of Attica have very little in common, as to origin, with the podzolic soils.

The phenomenon of podzolization of the red-tinted soils of Middle Europe indicates their ancient origin.

From our studies of the Greek red soils in the vicinity of Salonica we can indicate their age by their salinity. This process of salinity goes on in the red soils situated on terraces surmounting the valley of the Vardar River. This valley was occupied during the diluvial period by a very large salt lake (3). During this period the ground-water level was higher in the region of these terraces; only under similar conditions could the rise of salts have taken place. As a result of this process spots of solonetz and degraded solonetz soils are found up to this day.

The saturation of this type of red soils with sodium originates with the predominance of the eluvial processes. We may therefore observe a compact impervious illuvial horizon, enriched with organic matter and  $R_2O_3$  which are normally found on the lighter leached eluvial horizon. This phenomenon is reversed for the red soils of Attica that are not saturated with sodium.

The "solodi" varieties of red soils possess an acid reaction and in their morphology resemble the feeble podzolic brown earth of Ramann but have an entirely different origin. On this account it is not surprising to note that such soils had been mapped as brown earths.

We think that the study of the cycles of erosion in the Mediterranean region, and in Attica in particular, can disclose with a higher degree of accuracy the age of the red soils than of those that underlie the parent rock.

As has already been stated, the investigation of the origin and properties of

red soils is a very complicated task, in as much as it requires the study of factors that present considerable difficulties.

It seems to us that considerable difference of opinion as to the origin of the red soils is due to the absence of systematic studies of the Mediterranean region. This difference of opinion is accounted for by climatic conditions, topography, erosion, saturation processes with sodium, solodi and podzolic processes. The last condition can take place in regions of abundant rainfall (in some Mediterranean regions this reaches 800 mm.) and in regions situated high above sea level. The variety of conditions noted seemingly causes variation in the character of the red soils, not only in different latitudes and altitudes, but even within the boundaries of a small region whose soil-forming history is very old.

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# PLATE 1

FIG. 1. Profile of Agios Andreas

FIG. 2. Profile of Halandri

FIG. 3. Profile of Moravian Karst (Podzolic Soil)



FIG. 1

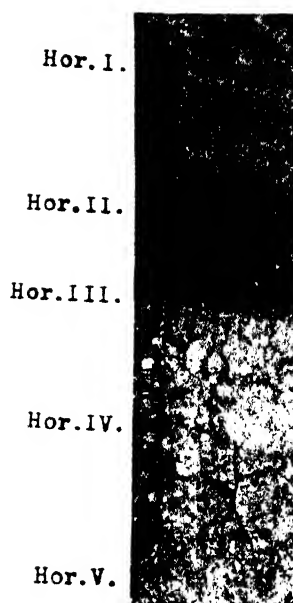


FIG. 2

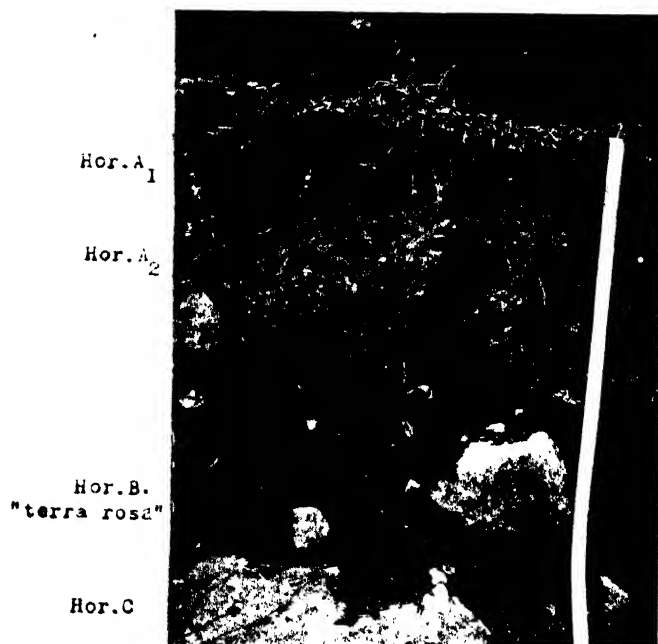


FIG. 3



## PLATE 2

FIG. 1. Concretions in the Profile of Agios Andreas

FIG. 2. Remnants of Limestone in the Profile of Agios Andreas

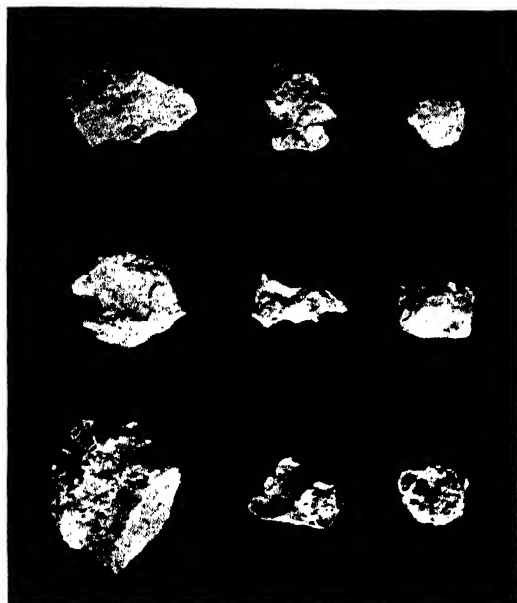


FIG. 2



FIG. 1



# THE COMPOSITION AND IONIC EXCHANGE OF FERRIC SILICATES AND PHOSPHATES<sup>1</sup>

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Since Way (18) demonstrated the base exchange capacity of soils, many workers, especially Wiegner (19), Hissink (7), Gedroiz (4), Kelley and Brown (9), and Mattson (10), have contributed to the general knowledge of the subject. It is evident, however, that there is a lack of unanimity as to the true mechanism of the reaction, partly because the composition of the colloidal anion is unknown.

It has been generally supposed that the acidoid component of the soil colloid is composed chiefly of silica and phosphoric acid, the part played by the hydroxyl group being usually neglected. Both the phosphate and the silica are combined with ferric iron or alumina, which forms the basoid part of the giant molecule, although other elements are present in smaller amounts. The composition of the soil colloid is extremely complicated, but a study of silicates and phosphates in their simplest form would materially aid in elucidating analogous phenomena such as occur in the soil.

## EXPERIMENTAL

*Preparation of ferric silicates.* A normal solution of ferric chloride was titrated against a solution of sodium silicate of known silica content until the iron was completely precipitated, which occurred at pH 5.4–5.6. To obtain precipitates of lower  $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$  ratio, the amount of sodium silicate was progressively reduced, the amount of reduction being made up with *N* NaOH until a pH of 5.4 was again reached. To obtain precipitates of high  $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$  ratio, the amount of sodium silicate was kept constant, but the ferric chloride was reduced, the diminished acidity being made up with *N* HCl to bring the whole, when mixed, to pH 5.4. By means of preliminary titrations the exact amounts of reagents required to fulfill the aforementioned conditions were determined.

The solutions were mixed by rapid pouring from one beaker to the other, and the floc was allowed to settle for one hour and was then filtered. To

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

prevent extensive hydrolysis, the precipitates were washed free of chlorine with 95 per cent alcohol; the residual alcohol was removed by drawing a current of air through the filter.

*Base exchange.* Two samples of the precipitate prepared as described were weighed. The dry matter was determined on one of them at 105°C. The base exchange capacity was determined on the other by leaching with *N* Ba-acetate (pH 7.0) until the pH of the leachate was equal to the pH of the original Ba-acetate. Ten cubic centimeters of *N* BaCl<sub>2</sub> was then added to the filter, and the precipitate was washed free of chlorine with water. The adsorbed barium was subsequently replaced with *N* NH<sub>4</sub>Cl, and the base exchange capacity was calculated to a constant weight of the dry material.

*Exchange neutrality.*<sup>2</sup> A 1 per cent suspension of the colloid was prepared, and 50 cc. of the suspension was added to 100 cc. of a mixed solution of sodium sulfate, sulfuric acid, and sodium hydroxide contained in a 200-cc. flask. The sulfate concentration was kept constant, and the total volume of colloid and salt solution was made up to 200 cc. The quantity of sulfate adsorbed was taken as the difference between the sulfate content of the original solution and that of the supernatant liquid. The pH after attainment of equilibrium was determined on the suspension with the quinhydrone electrode, and the pH of the salt solutions was determined at identical dilutions colorimetrically.

*Ferric phosphates.* The procedure adopted for the precipitation of ferric phosphates was identical with that used for the silicates. A solution of normal sodium hydrogen phosphate completely precipitated the iron from a ferric chloride solution at pH 4.3. By substituting sodium hydroxide for sodium phosphate the quantity of phosphate in the precipitate was varied as in the case of silicates.

#### DISSOCIATION OF AMPHOTERIC ELECTROLYTES

All amphoteric electrolytes are defined as compounds that possess both acid and basic properties and that on dissociation provide both hydrions and hydroxyl ions. An alternative suggestion has, however, been made, that is, that an amphoteric electrolyte is a compound that can both combine and provide hydrions. For a discussion on the definition of acids and bases the reader is referred to Davies (2), but the following discussion of amphoteric electrolytes is paraphrased from the work of Michaelis (12, p. 61-66):

If the ampholyte is present in solution in concentration  $[A]$ , as an acid it forms anions of  $[A^-]$  concentration, and its acid dissociation constant is  $k_a$ . It can also function as a base of dissociation constant  $k_b$ , and forms cations of  $[A^+]$  concentration. The ratio of the undissociated portion to the total concentration  $[A]$  of the ampholyte is given by the expression:

$$\rho = \frac{[A] - [A^+] - [A^-]}{[A]}$$

<sup>2</sup> This term is used by the authors in a sense not strictly comparable to that used by Mattson, who originated this term. In our case the displaceable cations or anions have not been removed by electrodialysis.

The degree of dissociation must be given separately for the cations and anions and is

$$\alpha^- = \frac{[A^-]}{[A]} \text{ for the anions, and } \alpha^+ = \frac{[A^+]}{[A]} \text{ for the cations.}$$

From the law of mass action

$$[A^-] \times [H^+] = k_a \times [U] \quad (A)$$

where U is the undissociated portion of the ampholyte, and furthermore,

$$[A^+] \times [OH^-] = k_b \times [U] \quad (B)$$

It follows, therefore,

$$A^- = \frac{k_a [U]}{[H^+]}$$

$$A^+ = \frac{k_b [U]}{[OH^-]}$$

and

$$[A] - [A^+] - [A^-] = U = [A] - k_a \frac{[U]}{[H^+]} - k_b \frac{[U]}{[OH^-]}$$

From which it follows

$$U = \frac{[A]}{1 + \frac{k_a}{[H^+]} + \frac{k_b}{[OH^-]}}$$

and hence the sought value of  $\rho$  may be stated as

$$\frac{[U]}{[A]} = \rho = \frac{1}{1 + \frac{k_a}{[H^+]} + \frac{k_b}{[OH^-]}}$$

Substituting for  $[OH^-]$  its value as  $\frac{k_w}{[H^+]}$  we obtain:

$$\rho = \frac{1}{1 + \frac{k_a}{[H^+]} + \frac{k_b}{k_w} [H^+]}$$

The maximum of the  $\rho^-$  curve can most easily be calculated as the minimum of the inverse function

$$\frac{1}{\rho} = 1 + \frac{k_a}{[H^+]} + \frac{k_b}{k_w} [H^+]$$

and

$$\frac{d}{d[H^+]} \frac{1}{\rho} = -\frac{k_a}{[H^+]^2} + \frac{k_b}{k_w}$$

and

$$[H^+] \text{ for } \rho = \text{max.} = \sqrt{\frac{k_a}{k_b} \cdot k_w}$$

The isoelectric point  $I$  is, therefore, at that  $[H^+]$  which is determined by the equation

$$I = \sqrt{\frac{k_a}{k_b} \cdot k_w}$$

The most important characteristics of the isoelectric point are: 1. At the isoelectric point the sum of the anions and cations of an ampholyte in a given concentration is at a minimum. 2. The concentration of the anions of an ampholyte at its isoelectric point is equal to that of the cations.

These conditions can be proved from equations  $A$  and  $B$ .

$$[A^-] = \frac{k_a \cdot [U]}{[H^+]} \text{ and } [A^+] = \frac{k_b \cdot [U]}{[OH^-]}$$

By substituting for  $[H^+]$  its value at the isoelectric point, and for  $[OH^-]$  the corresponding value

$$\frac{k_w}{[H^+]} = \frac{k_w}{\sqrt{\frac{k_a}{k_b} \cdot k_w}} = \sqrt{\frac{k_b}{k_a} \cdot k_w}$$

we obtain

$$[A^-] = [U] \cdot \frac{k_a}{\sqrt{\frac{k_a}{k_b} \cdot k_w}} \text{ and } [A^+] = [U] \cdot \frac{k_b}{\sqrt{\frac{k_b}{k_a} \cdot k_w}}$$

Therefore  $[A^-] = [A^+]$

There are, however, two large groups of ampholytes: those in which the acidic and basic radicals are spatially apart, such as the amino acids and proteins, and those in which the two radicals are one and identical, such as the metal hydroxides. Michaelis assumes the formation of an inner salt to explain the amphoteric behavior of the former, whereas the dissociation of a hydroxyl ion on the one hand and a hydrion on the other is sufficient to explain the amphoteric behavior of the latter. The strength of the ampholyte as an acid or base is determined by the ratio of  $\frac{k_a}{k_b}$ , but all acids or bases are probably amphoteric to a greater or lesser degree.

#### REPLACEMENT OF $SiO_2$ AND $PO_4$ BY THE $OH$ ION

The composition of the silicates varies with the proportion of the salts taken. With the ferric phosphates it was found impossible to prepare a compound of  $\frac{PO_4}{Fe_2O_3}$  ratio higher than 0.71, for the compounds decomposed until this ratio was formed.

In a previous discussion on phosphates by Mattson (10) and the senior author (14), emphasis was laid on the fact that for the precipitation of phosphatic compounds a certain concentration of hydroxyl ions is required to prevent acid hydrolysis. The requisite concentration depends on the nature of the compound, being higher for aluminum phosphate than for ferric phosphate.

At a certain pH range the precipitation of phosphate was increased by increasing the pH, but when the pH was further increased, the phosphate content of the precipitate was reduced, the  $\text{PO}_4^{=}$  being replaced by  $\text{OH}^-$ . The pH of precipitation in these experiments was originally chosen in order to give a compound of maximum insolubility, as denoted by the complete precipitation of iron. All the compounds were precipitated at this pH, but varied in composition, as sodium hydroxide was used instead of sodium silicate.

This relationship between the concentration of the silicate solution and the molecular ratio of the precipitate is perfectly linear, and so also is the rela-

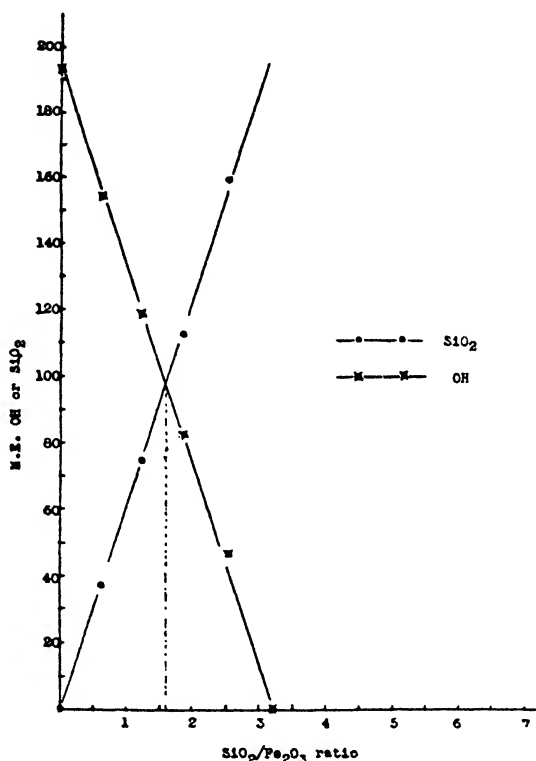


FIG. 1. RELATION BETWEEN  $\text{OH}$ ,  $\text{SiO}_2$ , AND COMPOSITION OF FERRIC SILICATES

tionship between the concentration of hydroxyl ions and the composition of the precipitates (fig. 1). There is another fact that is apparent, that is, the slopes of the lines are identical in both cases, but the graph connecting the concentration of hydroxyl ions with the composition of the precipitate is an exact mirror image of the graph connecting the concentration of the silicate ions with the composition of the same precipitate. This interrelationship is also apparent with the phosphatic compounds, where the graphs connecting the concentration of  $[\text{OH}^-]$  and  $[\text{PO}_4^{=}]$  ions with the molecular ratio of  $\frac{\text{PO}_4}{\text{Fe}_2\text{O}_3}$



of the precipitates are exact mirror images (fig. 2). Consequently, the hydroxyl ion is replacing the  $[\text{SiO}_3^-]$  and  $[\text{PO}_4^-]$  ions in exactly equivalent proportions. Silicates and phosphates can therefore be regarded as substituted hydroxides, a fact which is of great importance for the interpretation of base exchange.

$$\frac{\text{THE SiO}_2 \text{ RATIO}}{\text{Fe}_2\text{O}_3}$$

The composition of the clay complex of the soil varies with its stability under climatic conditions and the reaction of the medium. According to Mattson (10) the composition of the colloid and its pH so strongly influence each other that it is difficult to distinguish between the cause and the effect. Of particular interest from the viewpoint of the chemistry of silicates are the laterites

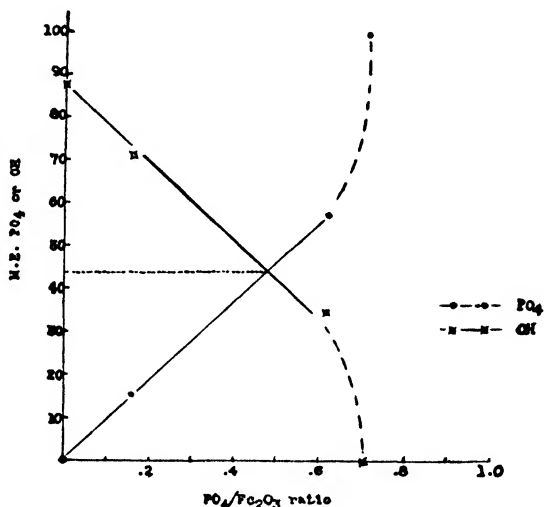


FIG. 2. RELATION BETWEEN OH, PO<sub>4</sub>, AND COMPOSITION OF FERRIC PHOSPHATES

and podzols, the chemistry of development of which has been discussed by numerous workers such as G. W. Robinson (16), Jones and Wilcox (8), and Reifenberg (15).

The development of both podzols and laterites can well be interpreted from the behavior of the ferric silicates. Although it is true that alumina is the chief basoid component of soils, there is always a high percentage of iron, and the behavior of aluminum and ferric silicates is so similar that what applies to one can also apply to the other. The ferric silicates, whatever the concentration of silica, could not completely precipitate the iron until pH 5.4 was reached. This is the synthetic counterpart of acid hydrolysis or podzolization. By maintaining the concentration of ferric chloride constant and precipitating the iron at pH 5.4 with a mixture of silica and hydroxyl ions, so that the product  $[\text{OH}^-][\text{SiO}_3^-]$  in equivalent concentration is always constant, a series of com-

pounds is obtained with varying quantities of silica as shown in figure 1. This is the synthetic equivalent of laterization where the silica can be replaced in part or completely by hydroxyl ions until ferric hydroxide is formed.

Mattson (10), in a theory of isoelectric weathering, put forward the suggestion that amphoteric soil colloids alter their composition in such a way that their isoelectric points coincide with the prevailing pH. In laterites under conditions of high rainfall the bases are dissolved; consequently, the prevailing pH initially will be alkaline and will become acid as the leaching of the bases continues. Since bases are again returned to the soil in considerable quantities by the plants, there are alternations of alkalinity and acidity in these soils. Under alkaline conditions ferric silicates are not stable, the hydroxyl ion replacing the  $\text{SiO}_2$ . The two compounds which originate by hydrolysis and are stable under such conditions are ferric and aluminum hydroxide, the isoelectric points of which when prepared from their chlorides are at pH 7.1 and pH 8.2 respectively. This theory gives a reasonable explanation for the exist-

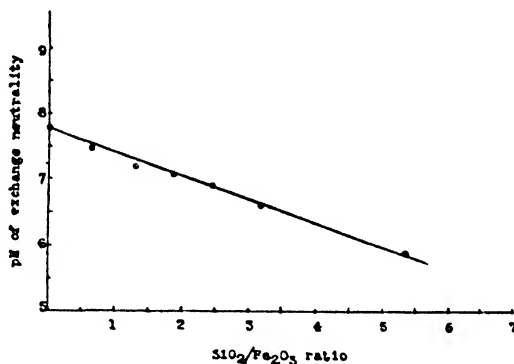


FIG. 3. RELATION BETWEEN  $\text{SiO}_2/\text{Fe}_2\text{O}_3$  RATIO AND pH OF EXCHANGE NEUTRALITY

ence of the hydrous oxides of both iron and aluminum in the free state in these soils.

The relationship between the composition of the precipitates and their pH of exchange neutrality is shown in figure 3. With increasing silica content of the precipitate the lower becomes the pH of exchange neutrality, which represents the most stable pH zone for that compound. At pH 7.8 the most stable compound formed with ferric iron in the presence of  $\text{SiO}_2$  and  $\text{OH}$  ions is ferric hydroxide.

There are cases, however, in soils of the lateritic type where there is considerable evidence of the mobility of the sesquioxides, which is attributed by Campbell (3) to the alkalinity of the ground water and by Harrassowitz (5) to the protective effect of silicic acid sols. The fact that in some lateritic soils there is considerable accumulation of free sesquioxides does not mean that they are stable under all conditions under which typical laterites are formed. The zone of precipitation of these hydroxides with respect to pH is extremely

narrow, and they are readily soluble in an excess of acidity or alkalinity as compared with their isoelectric pH. Secondary to desilication, this effect may also proceed, leading to a rather complete destruction of the surface layer in lateritic soils.

#### EXCHANGE NEUTRALITY AND ADSORPTION OF SULFATE WITH FERRIC SILICATES AND PHOSPHATES

The fact that soil colloids, phosphates, and silicates are amphoteric in behavior leads to both exchange acidity and exchange alkalinity. Both are manifestations of the dissociation of a colloidal electrolyte; in the former case the dissociated hydron is replaced by the cation of the salt, and in the latter

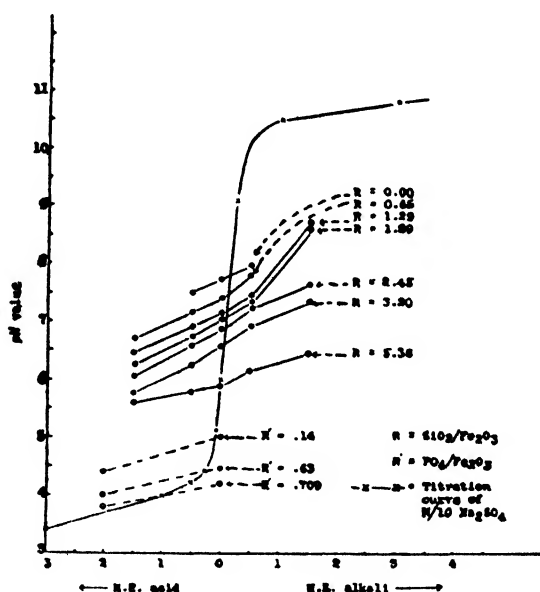


FIG. 4. EXCHANGE NEUTRALITY OF FERRIC SILICATES AND PHOSPHATES

the dissociated hydroxyl ion is replaced by the anion of the salt. This has been found for a large number of compounds by Mattson and Hester (10) and for hydroxides by the senior author (14). The graphs for the exchange reactions with these compounds, using a sodium sulfate solution of varying pH but of constant normality with respect to the sulfate anion, are shown in figure 4.

As has previously been found by Mattson (10), the point of exchange neutrality is consistently displaced to the acid side as the silicate or phosphate content of the precipitate is increased, the replacement of the hydroxyl by the phosphate ion increasing the acid dissociation, a new compound being formed by substitution with a stronger acidic but a weaker basic dissociation.

A remarkable regularity is observed in the adsorption of sulfate by both the

silicates and the phosphates (figs. 5 and 6). Ferrichydroxide has a considerable capacity for sulfate adsorption, but with silicates and phosphates the anion adsorption is greatly diminished, and with silicates of  $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$  ratio 1.89 it is non-existent, although they exhibit marked exchange neutrality. Each

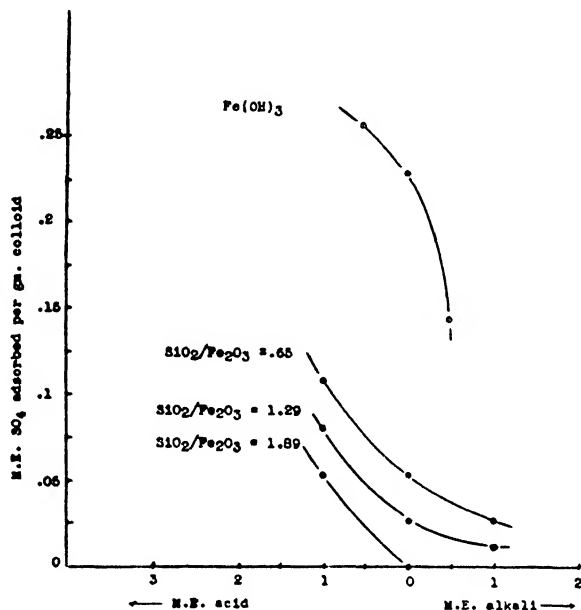


FIG. 5. SULFATE ADSORPTION BY FERRIC SILICATES

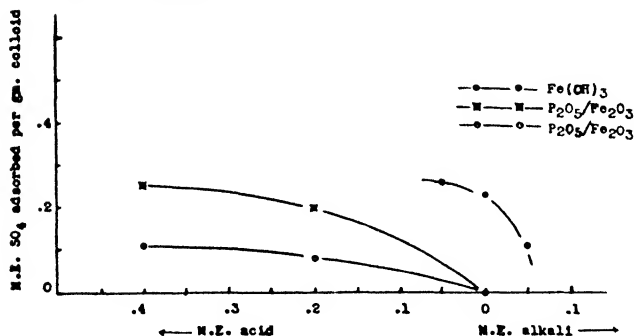


FIG. 6. SULFATE ADSORPTION BY FERRIC PHOSPHATES

component in the colloid influences the dissociation of the other, and increasing the phosphate or silicate content of the colloid by replacing the hydroxyl group appears to be accompanied by a diminished capacity for anion adsorption. As a function of the pH and the isoelectric point, both hydrions and hydroxyl ions are dissociated from the surface of the colloid, but other ions need not of necessity be adsorbed.

## BASE EXCHANGE CAPACITY OF FERRIC SILICATES

The base exchange capacity of the ferric silicates determined as previously described is represented graphically in figure 7. The base exchange capacity increases, as found by Mattson (10) with increasing ratio  $\frac{\text{acidoid}}{\text{basoid}}$ , reaches a maximum, and then diminishes.

It has been customary in base exchange studies to regard the colloidal fraction of the soil, whether organic or inorganic, as the seat of base exchange reactions, and to consider that the colloids themselves function as weak acids.

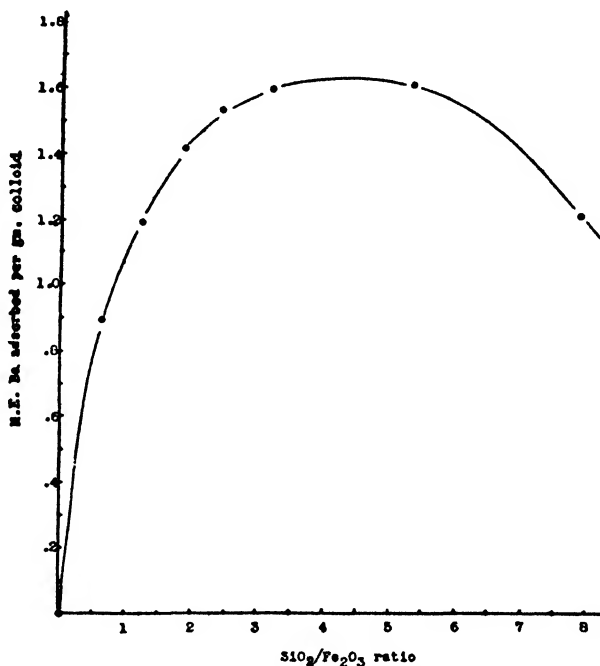


FIG. 7. BASE EXCHANGE IN RELATION TO  $\text{SiO}_2/\text{Fe}_2\text{O}_3$  RATIO AT pH 7

Bradfield (1) has suggested that the best method for determining the intensity or the strength of the acids is by the use of some expression analogous to the dissociation of weak acids, which is obtained from the mass action law and is given the expression

$$\text{pK} = \text{pH} + \log \frac{\text{salt}}{\text{acid}}$$

At the point of half neutralization the last term becomes 0 and the apparent pK value is numerically equal to the pH value at that point.

Wiegner (19) represents the particle of the inorganic soil colloid as if it were a crystal, but the double layer surrounding the micelle is composed of

two swarms forming an inner and outer layer of hydroxyl and hydrogen ions. If the outer layer is composed of hydroxyl ions, then exchange alkalinity is supposed to take place with a salt solution, but if the hydrions form the outer layer, then exchange acidity takes place, the hydrion being replaced by the cation of the salt.

The strength of ordinary acids at equivalent concentration can be determined with considerable accuracy from conductivity determinations, but this is only possible because the hydrogen ion has a much higher mobility than any anion. For amphoteric electrolytes, which, depending on conditions, dissociate the highly mobile hydroxyl ion as well, such a method cannot be applied, but the method of Michaelis of determining the isoelectric point with respect to the pH does give the requisite information and also a means of determining the relative strength of amphoteric compounds both as acids and bases.

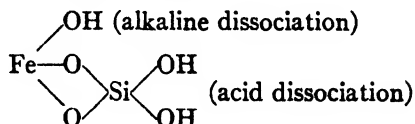
Regarding both silicates and phosphates as substituted hydroxides, as shown in figures 1 and 2, permits of a somewhat easier interpretation of both the origin of charge and the nature of base exchange reactions than was hitherto possible. It has previously (14) been stated that although hydroxides at their isoelectric pH have no base exchange, substituted hydroxides in soil, such as phosphates and silicates, if leached with a salt solution of the same pH, have base exchange under the same conditions, although below the isoelectric pH it becomes an absolute minimum. If these compounds are regarded as substituted hydroxides of the form  $X(\text{OH}) (\text{SiO}_2\text{H}_2)$ , such a phenomenon can be readily understood. If ferric hydroxide is treated with a solution of pH 7.2, there is in the solution a sufficient pressure of hydrions and hydroxyl ions to prevent the dissociation of either from the colloid. Even in substituted hydroxides the bond between the Fe and OH will be the same as in the pure hydroxide, but since some of the hydroxyl groups have been replaced by the acid  $\text{PO}_4$  and  $\text{SiO}_3$  groups, which dissociate hydrions from the surface, the substituted colloid will have a new isoelectric pH when the acid dissociation of the acid groups will be counterbalanced by the basic dissociation of the residual undisplaced hydroxyl groups. As more acid groups are introduced, the isoelectric pH of the colloid will be at a lower pH than that of a higher hydroxyl content, so as to permit an increased dissociation of hydroxyl groups to counterbalance the greater acid dissociation introduced by the substitution. Since both the dissociation of the hydroxyl and the acid dissociation of the other groups are a function of the pH, lowering the pH increases the former but suppresses the latter. With hydroxides both the acidic and basic dissociations arise from the dissociation of a single group  $[\text{X}-\text{O}-\text{H}]$ . With silicates, phosphates, and proteins the acidic and basic groups are spatially apart, but both are united directly with the central atom. An isoelectric point with no dissociation cannot therefore arise with a substituted hydroxide, for the hydroxyl group attached to the central atom is un-ionized only at the isoelectric pH of the hydroxide, but is ionized at a lower pH. The isoelectric point of a substituted hydroxide is at a pH where the concentration of hydrions and

hydroxyl ions is such that the dissociation of hydrions from the acid group balances the dissociation of the non-substituted hydroxyl ions.

With the ferric silicates under consideration, an extremely high ratio of  $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$  is required before the complete replacement of the hydroxyl groups is obtained, but with soils in which the ratio is much lower, the substitution is incomplete, and therefore most soils have an exchange alkalinity as well as an exchange acidity. The nearer the substitution of the hydroxyl groups by acid groups is to completion, the more likely the soil is to function completely as an acidoid without any perceptible basic dissociation, which is the case with soil colloids of extremely high silica content, such as bentonite. The higher the silica content of any silicate the higher therefore will be its saturation capacity at pH 7.0, provided that the stability of the compound is unchanged as the dissociated hydrions are replaced by the other cations.

In this respect silicic acid occupies a remarkable position. Electrokinetically, silicic acid always functions as an acidoid, but it is extremely unstable and breaks up into  $\text{H}_2\text{O}$  and  $\text{SiO}_2$  units. The  $\text{SiO}_2$  is an ion  $:\ddot{\text{O}}:\text{Si}:\ddot{\text{O}}:$  in which the oxygen has an excess of valency electrons or more than is required to balance the charge on its nucleus, and it has therefore a potential capacity for cation adsorption. Such a compound, however, is unstable, as, probably because of the unsaturation of the silicon atoms, the lone pairs of electrons of the oxygen are utilized in the polymerization of the  $\text{SiO}_2$  as described previously and are not available for the electrostatic attraction requisite for cation exchange.

With both iron and aluminum the lone pairs of the oxygen atoms of the silicic acid are utilized in saturating the iron and alumina, forming a compound of the following hypothetical structure:



By polymerization a micelle or crystal lattice is formed in which the lone electron pairs of the oxygen atoms of the silicic acid residue are utilized in saturating the iron and aluminum, leading to indefinite polymerization. The iron and aluminum are included, as previously described, in the micelle (14) but with hydroxyl groups at the surface. Since they are substituted hydroxides, a lower concentration of hydroxyl ions is required to form the micelle, and they are always formed and are stable at a lower pH than the hydroxides of the same metal.

With the silicates of high  $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$  ratio, although their points of exchange neutrality are at a low pH, they have under leaching conditions a tendency to

decompose at pH 7.0, some of the silica coming out of combination polymerizing on the surface of the ferric silicate, as does the free silicic acid, and preventing base exchange from proceeding. With the basic silicates, those of low  $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$  ratio, this does not occur, as sufficient ferric iron is present to utilize all the available linkages of the oxygen atoms of the  $\text{SiO}_2$ , until with increasing ratio more silicon is introduced so as to exceed the maximum co-valency of the iron. The point of maximum base exchange will therefore correspond to that point at which the silica can be used in saturating the iron, and further silicic acid introduced beyond that point gives an unstable combination. An attempt is being made to get a more stable acid than silicic or phosphoric in order to obtain a complete verification of this point.

Representing silicates as has been done above brings them into a class similar to the proteins as far as colloidal properties are concerned. Proteins are formed by the combination of amino acids, and silicates by the polymerization of simpler units, to form a molecule of colloidal dimensions. An aminic acid is formed by the substitution of an  $\text{NH}_2$  group for a hydrogen atom, and in a similar manner a silicate or a phosphate is formed by the substitution of silicic or phosphoric acid group for a hydroxyl group in a hydroxide in equivalent proportions. By continuous polymerization of the simpler units a micelle of colloidal dimensions is formed which can be broken up into simpler units by hydrolysis, but the iron and alumina can be in either the cationic or anionic parts of the hydrolyzed micelle until complete decomposition takes place.

Although Wiegner (19) represents the particle of a soil colloid with a double swarm of hydrions and hydroxyl ions, the authors find that such a conception does not permit of an adequate theoretical explanation to fit the observed facts, such as the influence and the significance of the pH of the leaching liquid and the isoelectric pH of the colloid itself on the saturation capacity. Michaelis' theory of dissociation as a function of the pH, however, does give the requisite interpretation. The fact that Michaelis derived his theory for soluble ampholytes makes no difference in its application to colloidal electrolytes which have hydroxyl groups at the surface. In both cases the dissociation can be regarded as a surface dissociation which is a function of the pH, and that one forms an insoluble micelle and the other is molecularly dispersed makes no difference as long as the dissociation is regarded as a surface dissociation which is a function of the pH.

The ionic exchange in silicates, phosphates, and hydroxides can be tentatively interpreted on the following bases:

Hydroxides, phosphates, and silicates attain the colloidal state by a continuous process of molecular polymerization with the formation of co-valent compounds.

Hydroxides have no base exchange if leached with a solution the pH of which corresponds to the isoelectric pH. When base exchange is taken as a test for ionization of the hydron, there can be no dissociation at the isoelectric point with hydroxides.

Substituted hydroxides have base exchange if leached with a solution the pH of which cor-



responds to their isoelectric pH, because the isoelectric point is reached when the dissociation of the residual undisplaced hydroxyl groups balances the acid dissociation of the silicic or phosphoric acid residue. The greater the proportion of silicic or phosphoric acid in the compound, the stronger the acid dissociation of the compound and the lower the isoelectric pH. Although there is dissociation at the isoelectric point with substituted hydroxides, it is at a minimum.

The dissociation of either hydroxyl or hydrions from the surface of the micelle is a function of the pH of the solution, and ionic exchange is a secondary reaction.

#### SUMMARY

Ionic exchange reactions have been studied with synthetic ferric silicates and phosphates. It has been shown that the adsorption of sulfate diminishes with increasing acidoid content of the colloid.

The ferric silicates have an optimum ratio at which the base exchange is a maximum.

The hydroxyl ion displaces the  $[\text{SiO}_3^-]$  and  $[\text{PO}_4^{=}]$  ions in equivalent proportions.

The application of the isoelectric point and the theory of amphoteric electrolytes to the interpretation of ionic exchange has been discussed.

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# A NEW APPARATUS FOR CARBONIC ACID ESTIMATIONS IN SOILS

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Researches have shown that  $\text{CaCO}_3$  in the soil performs a variety of functions of the greatest agronomic importance. For example, a serious displacement of calcium from soil colloids by H ions produces acidity, leading to conditions unfavorable for microorganisms as well as for cultivated plants. In arid regions the exchangeable calcium of the soil is often displaced by sodium to a considerable extent, resulting in the production of alkali soils. Moreover, a progressive removal of calcium from the exchange complex may result ultimately in lowering the base-exchange capacity of the soil. The degree of calcium saturation of the soil, together with the amount of a  $\text{CaCO}_3$  present, is also important in connection with the application of fertilizers and manures.

Gasometric estimations of carbonates in soils are generally made with the apparatus designed by Collins. Although convenient for many purposes, Collins' calcimeter suffers from certain inherent defects. The intervention of a rubber tubing between the reaction flask and the measuring burette may occasionally cause considerable errors. Moreover, since one end of the tube adjacent to the measuring burette is open to the air, the instrument is very sensitive to atmospheric pressure fluctuations. Further, the construction of the apparatus is complex, and a few rubber connections are inevitable. To circumvent these difficulties, a manometric apparatus for carbonic acid determinations was developed in this laboratory and has been in use for some time with satisfactory results. Some of the main advantages of this apparatus are as follows:

It is simple in construction and is easily manipulated.

With a view to precision in measurements, the use of rubber connections in the construction of the apparatus has been avoided.

It is independent of atmospheric pressure variations.

The use of a modified Thunberg tube (4) as the reaction vessel makes it possible to mix the reactants very effectively. Moreover, the detachable nature of this bulb, which can be shaken by hand apart from the apparatus, obviates the necessity of a shaking equipment.

## THEORY

The principle of the apparatus is the same as that employed by the authors for another purpose (3). The amount of  $\text{CO}_2$  evolved will be proportional to the increase of pressure in the reaction bulb. If  $h$  is the increase of pressure

in millimeters of Brodie solution<sup>1</sup> and  $x$  is the amount of  $\text{CO}_2$  evolved in  $\mu\text{l.}$  at N.T.P. (dry), we have

$$x = h.k$$

The value of the constant is calculated from the following simplified (2) formula:

$$k = \left( \frac{Vg \frac{273}{t + 273} + Vf. \alpha_{\text{CO}_2}}{P_o} + A \frac{273}{t + 273} \right),$$

where  $Vg = \mu\text{l.}$  gas in the bulb down to the zero mark on the manometric tube;

$Vf = \mu\text{l.}$  acid in side tube;

$\alpha_{\text{CO}_2} =$  Bunsen absorption coefficient of  $\text{CO}_2$  at  $t^\circ$ ;

$P_o =$  pressure of one atmosphere expressed in mm. of Brodie solution<sup>2</sup>

$A =$  area<sup>3</sup> of cross-section of manometer tube in sq. mm.

No thermostat is used with the apparatus, but a table is constructed giving the value of the constant at various temperatures from  $15^\circ$  to  $35^\circ\text{C.}$  By means of a sensitive thermometer placed in the water jacket, the temperature at which the experiment is being conducted is noted and the value of the constant given against it is utilized in the computation of volumes of  $\text{CO}_2$  evolved. After the amount of  $\text{CO}_2$  evolved in  $\mu\text{l.}$  at N.T.P. has been obtained, the percentage of  $\text{CO}_2$  in the soil is easily computed. One  $\mu\text{l.}$  of  $\text{CO}_2$  at N.T.P. weighs 0.001977 mgm.,<sup>4</sup> and the percentage by weight of  $\text{CO}_2$  in the original sample, say  $x$ , is given by the following formula:

$$x = \frac{V \times 0.1977}{w},$$

where  $V =$  volume of  $\text{CO}_2$  in  $\mu\text{l.}$  at N.T.P.

and  $w =$  weight of the soil sample in mgm.

#### DESCRIPTION OF THE APPARATUS

The apparatus (fig. 1) consists of a manometer (with a cross-sectional area not exceeding  $\frac{3}{4}$  sq. mm.), on one limb of which a 200-mm. scale is etched. The other limb of this manometer is blown into a bulb ( $P$ ) and carries a tap ( $T_2$ ) at its extremity. The circular mark ( $X$ ) below the bulb ( $P$ ) corresponds with the zero mark on the manometric scale. A screw-clamp arrangement with a

<sup>1</sup> The composition of Brodie solution is as follows: 500 ml. water, 23 gm. NaCl, 5 gm. sodium tauroglycocholate, and a few drops of an alcoholic solution of thymol.

<sup>2</sup> One atmosphere is approximately equivalent to 10,000 mm. Brodie solution.

<sup>3</sup> It should be clearly recognized that  $A$  must be less than  $\frac{3}{4}$  sq. mm. In case it greatly exceeds 1 sq. mm., errors of several per cent may be introduced under certain conditions by using the simplified formula given above.

<sup>4</sup> Since the density of  $\text{CO}_2$  is 1.529 referred to air as unity and 1 ml. of air at N.T.P. weighs 0.001293 gm., evidently 1 ml.  $\text{CO}_2$  will weigh 0.001977 gm. at N.T.P.

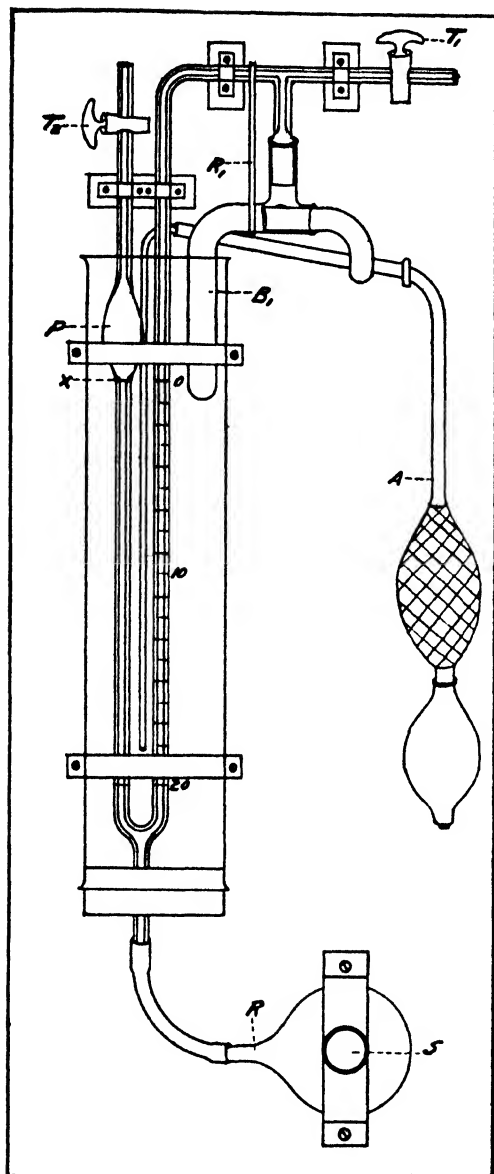


FIG. 1

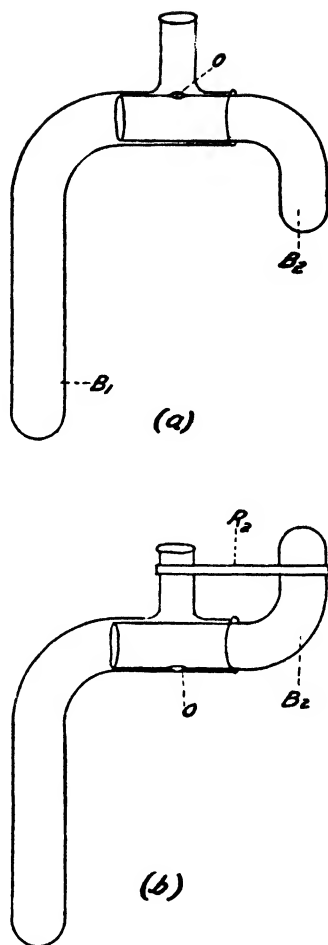


FIG. 2

FIG. 1 APPARATUS FOR CARBONIC ACID ESTIMATION IN SOILS  
FIG. 2 MODIFIED THUNBERG TUBE

rubber reservoir (*R*) is also provided, the manometric liquid being adjusted by means of a screw (*S*). The reaction bulb (fig. 2a) used is a modification of the Thunberg tube (4) and has a capacity of 30–35 ml. Through the tap (*T*<sub>1</sub>) the

pressure inside the manometer is equilibrated with the atmospheric pressure. The various parts of the apparatus are enclosed within a glass jacket, the water in which is kept agitated by means of the bellows (*A*) during experimentation. An accurate thermometer may be included in the jacket to record the temperature of the water. The whole outfit is mounted on a wooden stand for convenience in manipulation (plate 1).

#### METHOD OF USE

The soil is weighed and placed in the portion ( $B_1$ ) of the reaction bulb (fig. 2a) and the acid (HCl, 1:3), in the side tube ( $B_2$ ). As only small amounts of the soil are taken for the experiments, extreme care should be exercised in the preparation of the sample. Attempts should be made to have a representative and perfectly pulverized preparation (1). Usually 40 borings are made in each twentieth-acre plot, the samples from alternate borings being composited to make the duplicate samples. The quantity of soil and also of acid will depend upon the amount of carbonate in the soil, but 1 to 2 gm. of soil and 3 ml. of dilute HCl have been found to be convenient. Only small amount of  $\text{CO}_2$  should be allowed to be evolved, otherwise the gas may leak through the ground joint of the reaction bulb. With amounts up to 750 ml. there is absolutely no danger of leakage of  $\text{CO}_2$ . Anhydrous lanoline proved to be a suitable grease for the joints. When the side tube is being attached to the bulb it is advisable to apply 3 or 4 spots of the grease with a glass rod and then to press the side tube gently without rotating it. Under such circumstances the grease will flow uniformly over the ground surface, giving a thoroughly reliable joint. After the side tube has been fitted into the main part of the bulb (fig. 2a) the pressure inside the bulb is equilibrated with that of the air through the opening (*O*). Now the side tube is reversed, and a strong rubber band ( $R_2$ ) is carefully applied (fig. 2b). As a result of this reversal, the acid is spilled into the main part of the bulb, which is now violently shaken with the long limb held in a horizontal position. After being shaken for 4 to 5 minutes, the bulb is worked onto the ground joint in the manometer and is secured by a rubber band ( $R_1$ ). Now the taps ( $T_1$ ) and ( $T_2$ ) are opened, and the manometric liquid is adjusted to the zero mark on the manometric scale, when it should also stand at the mark (*X*) on the other limb. Subsequent to this adjustment, both the taps ( $T_1$ ) and ( $T_2$ ) are closed and the side tube ( $B_2$ ) of the reaction bulb is again reversed (fig. 1). Because of the release of the compressed gas in the bulb, the liquid level in the graduated limb will be depressed below the zero mark on the scale. The Brodie solution is again adjusted to the mark (*X*), and the reading on the manometric scale is noted. The reading so obtained represents the increase in pressure, referred to as *h* in the formula, from which the amount of  $\text{CO}_2$  evolved is easily computed. During experimentation the water in the jacket is kept stirred by means of the bellows (*A*).

## EXPERIMENTAL

The accuracy of the apparatus was tested by liberating known volumes of  $\text{CO}_2$  from bicarbonate solutions. The side tube received 0.2 ml. 0.05  $N$   $\text{H}_2\text{SO}_4$  which was added to an excess of bicarbonate solution contained in the main

TABLE 1

*Range of variability in  $\text{CO}_2$  evolved by 0.2 ml.  $\text{H}_2\text{SO}_4$  as measured by the apparatus*

$\text{CO}_2$ EVOLVED IN $\mu\text{l.}$ (N.T.P.)	DEVIATION FROM CALCULATED*	ERROR
		<i>per cent</i>
223.6	+1.0	0.45
220.4	-2.2	1.00
225.3	+2.7	1.25
222.0	-0.6	0.28
223.5	+0.9	0.40
224.5	+1.9	0.85
220.0	-2.6	1.20
221.5	-1.1	0.50
222.6	0	0
223.5	+0.9	0.40

\* Calculated amount of  $\text{CO}_2$  = 222.6  $\mu\text{l.}$  at N.T.P.

TABLE 2

*Range of variability in the carbonate content of a single sample as measured by the apparatus*

CARBONATE $\text{CO}_2$	DEVIATION FROM MEAN	ERROR
<i>per cent</i>		<i>per cent</i>
0.209	-0.002	1.0
0.212	+0.001	0.5
0.212	+0.001	0.5
0.213	+0.002	1.0
0.209	-0.002	1.0
0.208	-0.003	1.5
0.214	+0.003	1.5
0.213	+0.002	1.0
0.215	+0.004	2.0
0.208	-0.003	1.5
0.207	-0.004	2.0
0.212	+0.001	0.5
0.207	-0.004	2.0
0.209	-0.002	1.0
0.210	-0.001	0.5

part of the reaction bulb. The gas space inside the bulb was filled with a gas mixture containing 95 per cent nitrogen and 5 per cent  $\text{CO}_2$ . In order to fill it with a suitable gas mixture, the bulb is first exhausted by means of a water pump until the pressure gauge indicates the required pressure, after which the gas mixture is allowed into it. The capillary of the manometer need not be



filled with any gas mixture, as the total volume of the gas inside it is negligible as compared to the volume of the total gas space. The values obtained for  $\text{CO}_2$  evolved are presented in table 1. A glance at the values obtained will show that an accuracy of  $\pm 1.25$  per cent is easily obtainable in practice.

After the apparatus had been tested with bicarbonate solutions, some carbonate determinations in soils were made. In view of the great variations in carbonate content, it was thought advisable to make a number of determinations on a single sample of soil. The soil sample was obtained by digging a big hole to a depth of 1 foot and removing a uniform block of soil from the side. The soil was air-dried by placing it in a glass chamber, a current of air at 25–

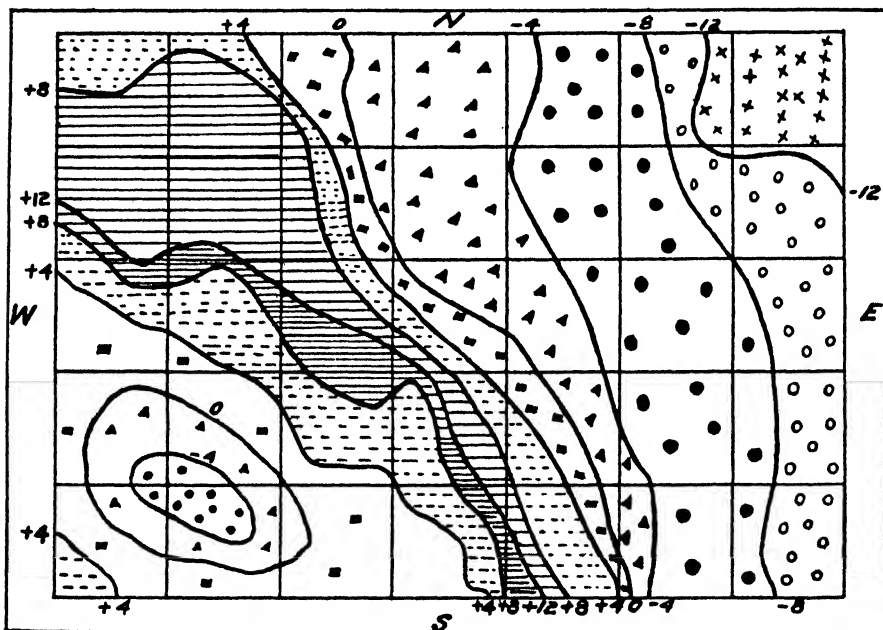


FIG. 3. CONTOUR MAP OF AN EXPERIMENTAL PLOT BASED ON THE CARBONATE CONTENT OF THE SOIL

30°C. being drawn over the soil by means of an electric fan. A mechanical analysis of the soil gave the following constituents: fine gravel, none; coarse sand, 0.7 per cent; medium sand, 0.6 per cent; fine sand, 1.8 per cent; very fine sand, 26.9 per cent; silt, 51.8 per cent; and clay, 18.2 per cent. One gram of air-dry soil was taken in the main part of the bulb while the side tube received 2.5 ml. dilute HCl. The values obtained for carbonate  $\text{CO}_2$  are given in table 2. A point which emerged from this and other similar analyses is that considerable variations occur in the carbonate content of the soil even within short distances.

To get an idea of soil heterogeneity with regard to  $\text{CaCO}_3$ , 875 carbonate estimations were made on samples obtained from a piece of land 140 feet by

100 feet. This field was divided into 35 plots 20 feet by 20 feet, each of which was again subdivided into 25 subplots 4 feet by 4 feet each. Borings were made about the center of the ultimate plots, and the samples were analyzed for carbonate  $\text{CO}_2$ . The points at which the carbonate content was 4, 8, or 12 per cent below or above the mean value for the whole field were joined, and the contour map shown in figure 3 was constructed. Generally speaking, variations in the carbonate content may exist from one corner of the field to another corresponding roughly with the line of slope or as random patches of higher or lower carbonate content. From the topography of the field under observation it became evident that most of the variations in carbonate content could be attributed to the leaching action of water. The occurrence of random patches of higher or lower carbonate content suggested that considerable errors might be introduced in the results of varietal tests or in the response of varieties to different manurial treatments due to this type of soil heterogeneity.

#### SUMMARY

An apparatus for the estimation of carbonic acid in soils is described. In the construction of the apparatus, the use of rubber connections, which is likely to cause errors in various ways, has been avoided and an effective mixing of reactants rendered practicable. Because of its detachable nature, the reaction bulb is easily shaken by hand, and no shaking apparatus is required.

The accuracy of the apparatus, as found in practice, is  $\pm 1.25$  per cent.

A few experimental data are presented.

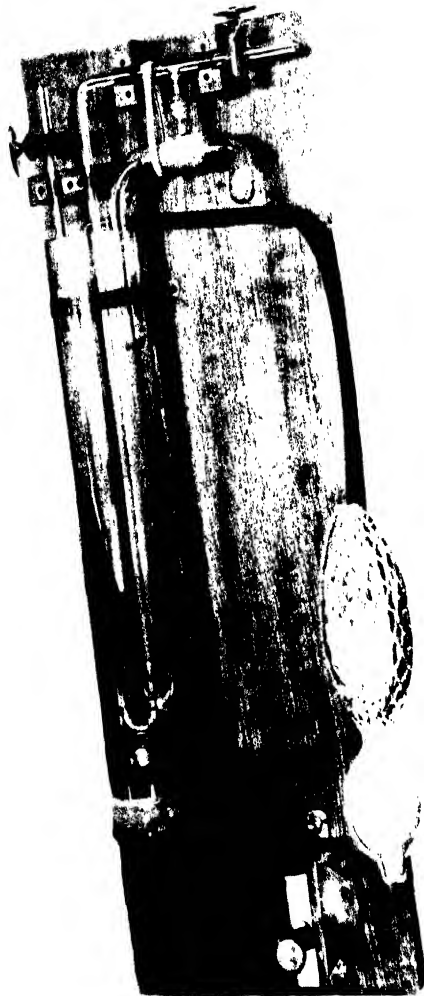
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PLATE 1  
APPARATUS FOR CARBONIC ACID ESTIMATIONS

CARBONIC ACID ESTIMATIONS IN SOILS  
R. N. SINGH AND P. R. MATHUR

PLATE I





# THE STATE IN WHICH THE HYGROSCOPIC MOISTURE EXISTS IN SOILS AS INDICATED BY ITS DETERMINATION WITH ALCOHOL<sup>1</sup>

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When air-dry soils are oven dried at 110°C. for 24 hours, a certain amount of water is driven off. It is not definitely known whether all this water exists in the soil as film or physically adsorbed water or whether part of it is also chemically combined or water of constitution. This uncertainty is especially true in the case of the organic portion of the soil.

It was thought that this question might be answered by determining the hygroscopic moisture of soils by means of alcohol. It was reasoned that if the moisture exists as physically adsorbed water or in the film form, the alcohol should extract or displace it. If, on the other hand, it exists as chemically combined water or water of constitution, the alcohol should not be able to extract or replace it.

In a former communication (1) it was shown that the total water content of soils can be accurately determined by means of methyl alcohol and a specially designed hydrometer. In that investigation it was found that the alcohol method agreed remarkably well with the oven-dry method in determining moisture content of soils. This agreement between the two methods would be a proof that all the water in the soil that is driven off at 110°C. exists in a physical form. In view of the fact, however, that the alcohol tends to dissolve some material from the soil, especially from the organic portion, and that this dissolved material affects the hydrometer reading in the same way as does water, it could not be concluded definitely that the alcohol takes out all the hygroscopic moisture. It was feared that any material dissolved by the alcohol might compensate for unextracted water.

In order to eliminate the difficulties presented by the hydrometer method, a reagent was required which would dissolve and separate the alcohol from the water and thus measure the water directly by volume. Such a reagent has been devised and patented by John F. Williams (2). It consists of a mixture of fusel oil, toluene, and tartaric acid. The fusel oil extracts the alcohol, the toluene prevents the fusel oil from retaining any water and separates and expels the water from the reagent, and the tartaric acid renders the reagent acid and

<sup>1</sup> Authorized for publication by the director as Journal Article 252 n.s. of the Michigan Agricultural Experiment Station.

thus prevents the formation of emulsions. When this reagent is mixed in a cylinder with ethyl alcohol containing water, two liquids separate out with a sharp line of demarcation between the two layers. The lower column of liquid is water, and the upper, a mixture of the other constituents. Actual tests have shown that this reagent will separate and determine the alcohol content of an ethyl alcohol-water solution within an accuracy of 0.5 per cent. With concentrations above 65 per cent alcohol the reagent does not work; such concentrations have to be diluted with distilled water. The presence of dissolved solids, except when the amount is rather large, does not affect the accuracy of the reagent.

To ascertain by the aid of this reagent whether or not the alcohol is capable of extracting all the hygroscopic moisture in soils and in some organic materials, the following procedure was devised. Into an Erlenmeyer flask were placed 50 gm. of air-dry soil and 50 cc. or more of 95 per cent ethyl alcohol. The flask was stoppered, and the mixture was allowed to stand for 24 hours with occasional shaking. The supernatant alcohol was then filtered and very accurately diluted with distilled water in the ratio of 1 to 1. Then 10 cc. of this liquid was taken by a 10-cc. pipette and placed in a special very narrow 25-cc. cylinder graduated into 0.1 cc. The cylinder was then filled with the reagent up to the 20-cc. mark, stoppered with a rubber stopper, and inverted seven or eight times to mix the reagent thoroughly with the alcohol solution. The cylinder was then set upon a table and allowed to stand for the water to separate from the mixture. At a temperature around 73°F. the separation of the water can be accomplished in about 10 minutes, but for certainty and high accuracy about 2 hours' time was always allowed for the separation. The volume of water so separated was carefully read and recorded. For basis of comparison or for ascertaining the exact amount of water that the alcohol extracted from the soil, a check was always run with the stock alcohol solution. This check test was performed exactly in the same way and at the same time as the test with the alcohol extract solution, and thus any error in the method was minimized or eliminated from the final experimental results. The difference in the volume of water between stock solution and the extract solution represents the amount of water contained in 10 cc. of each solution used. When this difference is multiplied by the total volume of alcohol employed to extract the soil, the total amount of water extracted by the alcohol from the soil is easily obtained. From the amount of soil used and the amount of water extracted from it, the percentage of water in the soil is readily calculated. This percentage of hygroscopic moisture as determined by the alcohol method is then compared with the percentage of hygroscopic moisture as determined by the oven-dry method in which the soil is dried at 110°C. for 24 hours.

The alcohol method as described has shown itself to be reliable and accurate, especially when checks are run parallel with the experimental tests and the tube is kept thoroughly clean and the temperature is above 70°F. At low temperatures the separation of the liquids is slow and frequently incomplete.

In table 1 are shown the percentages of hygroscopic moisture as determined by the alcohol method and by the oven-dry method in a number of typical mineral and organic soils.

The experimental data in table 1 show that in the case of the mineral soils the hygroscopic moisture as determined by the alcohol method is the same as that determined by the oven-dry method, indicating that the alcohol can extract or replace all the hygroscopic moisture. It will be noticed that the variation in the two methods is less than 1 per cent, which is considered to be within the experimental error. In the case of the mucks and peats, however, the oven-

TABLE 1  
*Comparison between alcohol method and oven-dry method in determining hygroscopic moisture in soils*

MATERIALS	DEPTH	ALCOHOL METHOD	OVEN-DRY METHOD
	<i>inches</i>	<i>per cent</i>	<i>per cent</i>
Fuller's earth.....	....	9.2	10.0
Bentonite.....	....	10.57	11.31
Silica gel.....	....	1.40	1.04
Kaolin.....	....	1.20	1.16
McKenzie clay.....	B horizon	7.50	6.76
Grundy silt loam.....	Surface	2.50	2.74
Hagerstown clay loam.....	70-80	4.05	4.29
Hagerstown clay loam.....	12-70	2.90	2.81
Davidson clay loam.....	24-30	2.01	2.15
Greenville fine sandy loam.....	12-18	1.38	1.25
Decatur clay.....	0-6	1.20	1.32
Susquehanna clay.....	2-6	3.72	3.98
Lufkin clay.....	10-16	9.35	10.22
Colby silt loam.....	subsoil	1.74	1.85
Fargo clay loam.....	6-16	5.38	5.23
Copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ).....	....	0.00	37.8
Muck 1.....	....	10.1	15.2
Muck 2.....	....	10.66	15.7
Muck 3.....	....	9.17	16.2
Peat 1.....	....	9.00	13.35
Peat 2.....	....	9.98	15.02
Wheat flour.....	....	8.82	12.80

dry method gives about 5 per cent more moisture than the alcohol method, indicating either that the alcohol is unable to extract all the hygroscopic moisture or that the oven-dry method causes these organic materials to undergo partial decomposition which is revealed as hygroscopic moisture. The latter hypothesis is probably the correct one, inasmuch as the alcohol is able to extract the hygroscopic moisture of such materials as silica gel, fuller's earth, and bentonite, and the nature of the organic materials is such that some decomposition might be expected to take place at the temperature of 105-110°C.

All evidence obtained in the course of the present investigation indicates



that the data in table 1 are reliable and accurate. One of the strongest evidences that such is the case is afforded by the results in table 2, which show that after bentonite, fuller's earth, and various soils have been dried at 110°C. for 24 hours alcohol extracts no water or anything else which affects the reading, and yet when these materials are in air-dry condition considerable amounts of water are extracted.

The facts revealed in the experimental data in table 1 are of fundamental importance. They indicate (a) that the hygroscopic moisture in the mineral soils probably all exists as physically adsorbed or film water; (b) that even though this film water is held with enormous forces, is greatly compressed, and can be expelled only at a temperature of 110°C. for several hours, yet the alcohol is able to extract it readily in only a few minutes; (c) that in the case of the organic materials a considerable part of the loss on heating at 105°–110°C. may represent volatilization and water of decomposition and not hygroscopic moisture; (d) that the method previously offered (1) to determine the moisture

TABLE 2  
*Amount of water extracted by alcohol from soils in oven-dry and air-dry condition*

SOILS	OVEN-DRY SOILS	AIR-DRY SOILS	MOISTURE DETERMINED BY OVEN-DRY METHOD IN AIR- DRY SOILS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Bentonite.....	0.0	10.57	11.31
Fuller's earth.....	0.0	9.20	10.00
Muck.....	0.0	10.20	15.2
Clay.....	0.0	7.50	6.76
Loam.....	0.0	4.05	4.29

content of soils by means of alcohol and a hydrometer is sound in principle and reliable.

Attention must be directed to the results (table 1) obtained with copper sulfate, a hydrate which is insoluble in alcohol. It will be seen that this copper sulfate contained 37.8 per cent water, according to the oven-dry method, and yet the alcohol failed entirely to extract any of this water. These results are suggestive and significant as to the different forms of water which may exist in the materials.

#### SUMMARY

Hitherto it has not been definitely known whether the hygroscopic moisture of soils, which is driven off by heating at 110°C., exists exclusively as film or physically adsorbed water or partly as chemically combined water.

An attempt has been made to throw light upon this question by extracting soils with alcohol and then determining the quantity of water found in the alcoholic extract. By means of a special reagent consisting of fusel oil, toluene,

and tartaric acid, the water extracted by the alcohol can be determined volumetrically.

It was found that in the case of mineral soils, the alcohol extracts all the hygroscopic water, indicating that the hygroscopic moisture in mineral soils exists entirely in the form of physically adsorbed or film water

In the case of mucks and peats and some other organic materials studied, the alcohol extracts about one third less water than is indicated by the oven-dry method. The explanation for this is that either the alcohol is unable to extract all the water from the organic materials or the oven-dry method causes these organic materials to undergo partial decomposition or volatilization of substances other than water, with consequent high and erroneous values for hygroscopic water. The latter seems the more likely possibility.

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# THE INFLUENCE OF THE REACTION OF SOIL STRATA UPON THE ROOT DEVELOPMENT OF ALFALFA<sup>1</sup>

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Failure in the growth of alfalfa is experienced in many cases in the eastern part of the United States where apparently good stands and growth should be obtained, if one judges from cultural practices. In some instances where rather acid soils have been recently limed for the first time, it is observed that good stands are obtained, but the yield is low the second year and the alfalfa "runs out" quickly. Inspection of some of these cases reveals a shallow rooting system. In other instances it is noticed that soils of a similar nature which have been continuously and systematically limed over a long period of years produce good crops.

These observations have led to the study of the reaction of soil strata upon the root development of alfalfa.

## PLAN OF EXPERIMENT

The alfalfa in this experiment was grown at the Pennsylvania State College in 52 sewer tile, 15 inches in diameter and 24 inches in depth, placed in the ground up to the flange. Care was taken to provide good drainage, and clover was planted around the tile for protective purposes. The various treatments were made in replicas of six to allow a set of two to be removed in consecutive years.

### *Soil used*

The soil used was a virgin Dekalb silt loam surface soil obtained from the Pennsylvania Experiment Station substation at Snowshoe. None of the A<sub>0</sub> horizon was used. The pH value (quinhydrone) was 4.7.

### *Treatments*

Two main treatments of one-half lime requirement and full lime requirement were made to the surface 3 inches of soil. Each of these treatments had sub-layer treatments of no lime, one-fourth lime requirement, and one-half lime

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requirement. In addition, one set received one-half lime requirement to the depth of 6 inches with no lime to the sublayer, and one set had no lime added to either layer.

In all cases muriate of potash and superphosphate were added to the upper 3 inches of soil *at the rate* of 100 pounds and 600 pounds respectively, to the million pounds of soil. The various treatments, including the calcium carbonate treatment, are shown in the following tabulation.

LIME TREATMENT*		CaCO <sub>3</sub> PER 1,000,000 POUNDS MOIST SOIL	
0"-3"	3"-24"	0-3"	3"-24"
		pounds	pounds
0 L	0 L	.....	.....
1/2 L	0 L	1,000	.....
1/2 L	1/4 L	1,000	500
1/2 L	1/2 L	1,000	1,000
1 L	0 L	2,000	.....
1 L	1/4 L	2,000	.....
1 L	1/2 L	2,000	1,000
1/2 L†	0 L	1,000	.....

\* 0 L = No lime.

1/4 L = 1/4 lime requirement.

1/2 L = 1/2 lime requirement.

1 L = Full lime requirement.

† Limed six inches deep.

The lime applied was in the form of calcium carbonate testing 96 per cent CaCO<sub>3</sub> and 1 per cent MgCO<sub>3</sub>, 85 per cent passing through a 100-mesh sieve and all through a 30-mesh sieve.

The lime requirement was determined by replacement of the hydrogen with a normal BaCl<sub>2</sub> solution and titrating with NaOH.

### Cultural data

Grimm alfalfa was inoculated with an especially hardy strain of *Bac. radicola*. The alfalfa was thinned each year to the ten best plants. It was planned to permit the alfalfa to grow over a 2-year period, but winter killing necessitated replanting of the remaining tile the second spring. On the third series of tile this second year's planting was allowed to remain until the following spring of 1930.

The cultural record is as follows:

OPERATION	1928	1929
Put soil in tile.....	May 12-19	
Planted alfalfa.....	May 31	May 27
Sampled for replaceable Ca.....	Sept. 27	Aug. 11
Harvested tops.....	Oct. 1-11	Aug. 12-18
Removed roots.....	Oct. 1-11	Aug. 12-18

## SOIL CONDITIONS DURING THE EXPERIMENT

*Hydrogen-ion concentration*

The hydrogen-ion concentration of the surface soil for the unlimed tile was about 4.8 (quinhydrone) throughout the experiment; for the treatment receiving 1000 pounds of limestone to 1,000,000 pounds of soil, about 5.6; and for the surface soil receiving 2000 pounds of limestone to 1,000,000 pounds of soil, 6.3.

After the limestone was added May 12 to 19, 1928, the pH values of all the tile reached their highest value in the July 22, 1928, sampling (fig. 1). A slight decrease in pH value took place throughout the remainder of the season. In 1929 the pH value of the surface 3 inches on April 27 was slightly higher than on the preceding October. As in 1928, there was a slight decrease in pH as the season progressed. On the whole, the pH value of the surface soil for each treatment was lower the second year than the first year. This difference, however, was not great.

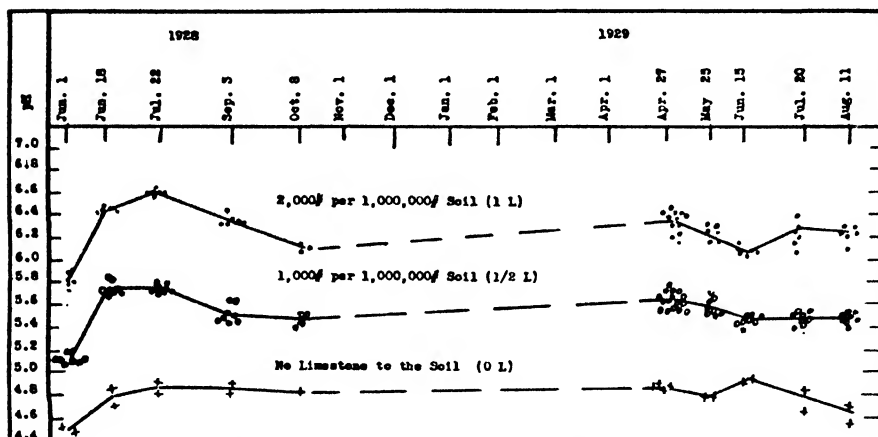


FIG. 1. pH OF SURFACE 3 INCHES THROUGHOUT SEASONS 1928-1929

A comparison of the pH values between years (fig. 2) in the various soil layers taken at the end of each season shows, in general, an essential similarity. A slight raise in pH value occurred in the second year in the 6- to 12- inch layer and possibly a slight lowering, in general, of pH in the first and second 3-inch layers. This change between years, however, in the most extreme case between different tile of the same treatments, was only a pH of 0.34 and, in the majority of cases, was less than 0.2 pH, which is within experimental error. Accordingly, a discussion of the average of the two years is all that is necessary at this place.

The soil in the tile receiving no lime treatment to either the surface or sub-layer had nearly the same pH value for both the upper and second 3 inches (pH 4.7).

The pH of the second 3 inches of the limestone treated tile, however, shows that it was affected by the quantity of limestone applied to the surface 3 inches

as well as that applied to the sublayer. Also, the higher quantities of limestone added to the various sublayers augmented this change. In general, the pH for the second 3 inches for the two years was half way between the pH values of the surface layer and the sublayer lying below the depth of 6 inches.

The pH of the unlimed sublayer below the depth of 6 inches was about 4.7, or the same as that for the tile receiving no limestone in the entire soil column. For the sublayer below 6 inches receiving 500 pounds of limestone ( $\frac{1}{2}$  L) the pH averaged 5.1, and for that receiving 1000 pounds of limestone, pH of 5.6, or the same as the surface 3 inches with the same treatment.

These results show that the surface treatment decreased the acidity of the second 3 inches to some extent and possibly a very small amount of the second 6 inches over a 2-year period.

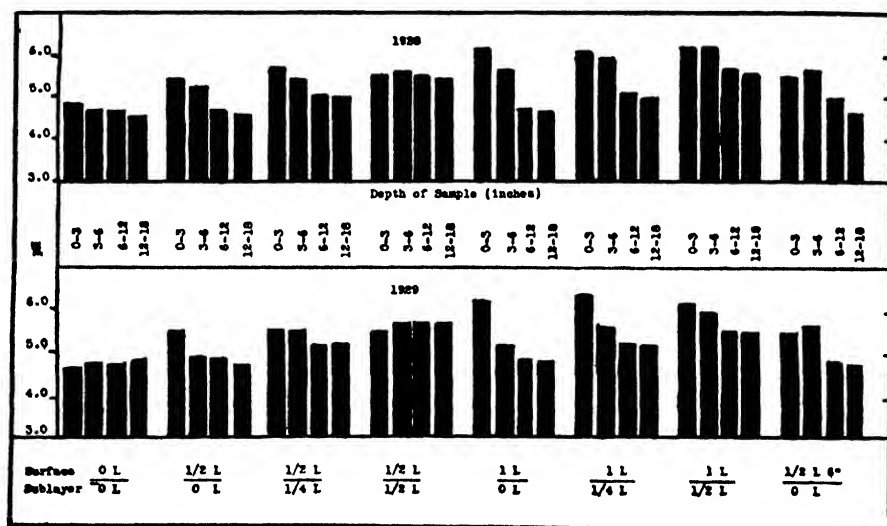


FIG. 2. pH OF THE SOIL AT THE DIFFERENT DEPTHS

### *Replaceable calcium*

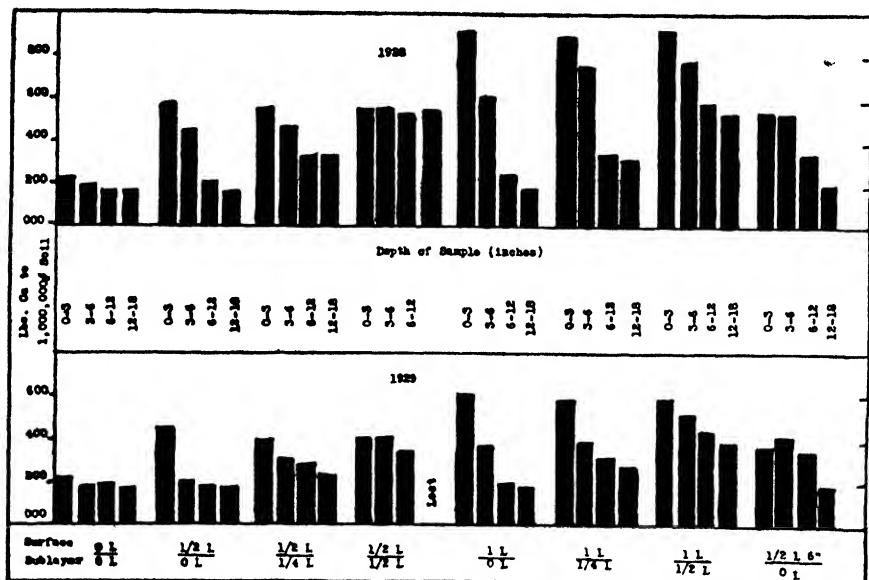
One of the outstanding results of the experiment is the close correlation between replaceable calcium and the hydrogen-ion concentration.

At the end of the first season the correlation coefficient between replaceable calcium at the various soil depths and the pH was  $0.974 \pm 0.00433$ . At the end of the second season it was  $0.923 \pm 0.01346$ . The correlation using all the values for both years was  $0.856 \pm 0.0165$ .

The replaceable bases were extracted with 0.1 N HCl, which may account partly for the poorer correlation when the two years are taken together. No doubt some of the calcium analyzed the end of the first season as replaceable was not strictly replaceable but came from  $\text{CaCO}_3$  remaining in the soil from the original application. Also, the surface 3 inches of all the tile received an application of 600 pounds of 16 per cent superphosphate to 1,000,000 pounds of soil,

which, especially the first year, is reflected in the exchangeable calcium in this layer.

Since there is so high a correlation between pH and replaceable calcium for each of the various depths at which the samples were taken, it is not surprising to find that the soil conditions in regard to replaceable calcium parallel those already discussed for pH (fig. 3).





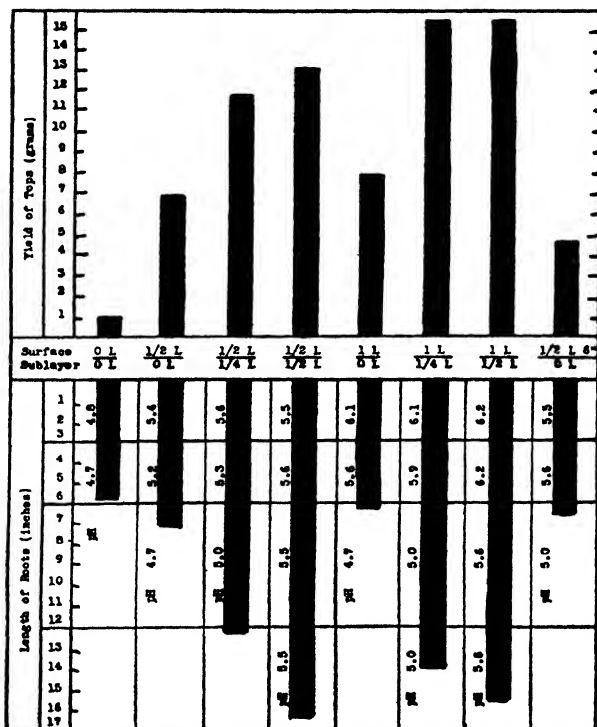


FIG. 4. RELATIONSHIP BETWEEN YIELD, ROOT GROWTH, AND pH VALUE, 1928

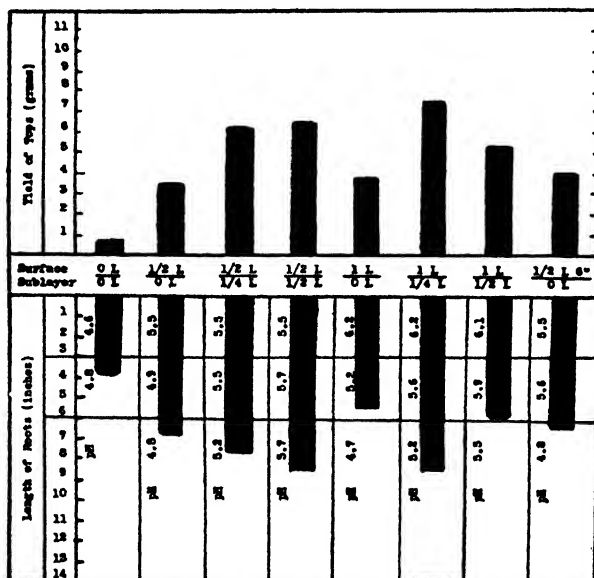


FIG. 5. RELATIONSHIP BETWEEN YIELD, ROOT GROWTH, AND pH VALUE, 1929



TABLE 1  
*Nodulation of roots at different depths for various treatments*

TREATMENT		1928		1929	
Surface	Sublayer	0-3"	3-6"	0-3"	3-6"
0 L	0 L	Poor	Poor	Poor	Poor
1/2 L	0 L	Medium	Fair	Fair	Fair
1/2 L	1/4 L	Good	Medium	Medium	Medium
1/2 L	1/2 L	Medium	Medium	Good	Medium
1 L	0 L	Good	Poor	Good	Medium
1 L	1/4 L	Good	Medium	Medium	Medium
1 L	1/2 L	Good	Good	Good	Medium
1/2 L 6"	0 L	Medium	Medium	Good	Good

TABLE 2  
*Conditions of plants in tile after winter of 1929-30*

TREATMENT		TILE NUMBER	NUMBER OF PLANTS		CONDITION
Surface	Sublayer		Fall December 20, 1929	Spring June 7, 1930	
0 L	0 L	C10	4	4	Very small
		D12	10	6	Very small
1/2 L	0 L	A9	4	4	Small
		B11	8	8	Small
1/2 L	1/4 L	B9	11	11	Fair
		C11	7	7	Fair
1/2 L	1/2 L	C9	10	10	Good
		D11	12	9	Good
1 L	0 L	C13	8	8	Fair
		D9	10	10	Fair
1 L	1/4 L	A10	8	8	Fair
		D13	8	8	Fair
1 L	1/2 L	B10	7	7	Good
		C12	12	7	Good
1/2 L 6"	0 L	A11	8	8	Fair
		D10	9	9	Fair

*Vigor of the plants the second spring*

The first year the plants were all winterkilled, but the second year planting did not fare so badly, no winterkilling being noticed after the winter of 1929-30.

The growth on the tile receiving no limestone was very poor the spring of 1930. That receiving only 1000 pounds to the surface and none below 3 inches also showed poor growths. The tile receiving 1000 pounds and 2000 pounds to the surface and 500 pounds to the sublayer showed fair growth. Tile having 2000 pounds and 1000 pounds added to the surface 3 inches per acre and 1000 pounds to the sublayer showed good vigor in the spring (table 2).

#### DISCUSSION

The results show that under the conditions of the experiment root growth was meager in the soil layers at a pH under 5.0, but that upon addition of lime to bring the pH to 5.5 and 6.2 good root penetration and subsequently good yields of tops were obtained. The results further show that applications of lime raise the pH and replaceable calcium in the immediate sublayer soon after addition of the limestone, which in turn influences the root growth.

The poor growth in soil layers having a pH of less than 5.0 may be attributed to one or a combination of the following factors: deficient nutrient supply, aluminum toxicity, high hydrogen-ion concentration.

#### *Deficient nutrient supply*

In this experiment, if root growth had been just poor or nominally weak in the layer receiving no lime, there might have been a question of the effect of nutrient supply on root development. The root growth in this experiment, however, was unusually weak, if not almost entirely inhibited, in the soil layers which received no lime. That sufficient food nutrients are present for general growth is shown by the natural vegetation on the soil in its native state.

Although Weaver, Jean, and Christ's (17) experiments show that where roots come in contact with a fertilized layer of soil they developed more abundantly, branched more freely, and the presence of such a layer seemed to retard normal root penetration into the soil below, they did not say it inhibited root growth in the other layers.

On the other hand, the works of Wolters (21) with cane, Lees (10) with wheat, and Davis (4) with alfalfa show that under normal conditions the roots of plants receiving fertilizers penetrated deeper than those receiving no fertilizers.

The work of Weller (18) with roots of cane in root study boxes with fertilizers applied to various parts of the root system is also of interest here. His results show that stimulation of the entire root system as well as a local stimulation was found to be the case.

Gile and Carreo (7) with different nutrient solutions of nitrogen, phosphorous, and potassium confined to separate roots showed that there is an unusual transference of nitrogen to the roots in phosphorous and potassium solutions and an extraordinary transference of phosphorous to the roots in the

nitrogen and potassium solutions. With other solutions which might represent the conditions of this experiment, they found the following:

TREATMENT		GRAMS PER ROOT	
Flask A	Flask B	Flask A	Flask B
N P K	O	0.0455	0.0267
N P K	K	0.0302	0.0205

The flasks, of course, contained the other necessary nutrients.

In considering separate nutrients, Brenchley (2) has shown that a provision of phosphates for the first 6 weeks allowed normal growth to take place in grain. Gericke (6) obtained a maximum dry weight with wheat when the plants were grown in the nutrient solution for 4 weeks and were then transferred to solutions containing no phosphate. The continuance of growth of the roots in these experiments shows that phosphorous is transferred to the root from one part to another.

Truog (16) further shows that "solid phase" feeding probably takes place in plant nutrition, especially with phosphorous. A pH of 4.7 or aluminum and iron phosphate should not interfere entirely with phosphorous assimilation. Furthermore, available phosphorous is never found except in traces in Dekalb soil such as was used in the experiment reported here. Still a good growth was obtained in the sublayers which received no phosphorous but were limed.

From this review of literature and the results obtained in the experiment reported in this paper, it is believed that the plants received sufficient phosphorous for root penetration in the soil layers with a pH below 5.0.

It has been thought by some that lack of calcium for nutritional purposes might account for the decrease in yield of alfalfa on soil low in calcium, but this will not account for lack of root penetration if we concede that calcium absorbed by one part of the root system is available to other parts of the root system, as is apparently the case with phosphorous. Also, it will be remembered that although the roots showed very good growth in the upper limed layer, they did not penetrate into the lower layer with a pH of 4.7.

Duley (5) reports that where the soil contained 553 pounds of calcium per acre soluble in 0.04 *N* carbonated water, liming was profitable, whereas where 810 pounds were soluble, only small returns from liming were obtained. Along this same line Whitson, Chapman, and Hull (19) found that with the extraction of calcium with 0.04 *N* carbon dioxide solution, soils showing but 346 pounds to the acre produced poor alfalfa whereas soils with 565 pounds produced good alfalfa. These results show that calcium as a food supply may be a limiting factor in growth.

The experiment conducted here, however, shows that representative soil

layers with varying pH values had the following amounts of 0.1 *N* HCl extracted calcium to 2,000,000 pounds of soil:

pH	Pounds of 0.1 <i>N</i> HCl extracted calcium
4.7	380
5.2	926
5.4	1,096
5.8	1,476
6.2	1,565

No doubt 0.1 *N* HCl extraction and 0.04 *N* carbonated water extraction are not comparable; however, judging from the root growth in the various horizons, the soil with a pH above 5 had sufficient calcium for plant growth. Under the conditions of this experiment the layers with a pH of 4.7 where little root growth took place had, perhaps, close to the limiting point of available calcium for plant growth. With large mature plants in this soil, calcium might have been a limiting factor if the plant had grown to this extent in the unlimed soil, but it is felt that at a pH of below 5.0 some factor other than calcium was the limiting factor. At a pH of above 5.0, where plant growth was more nearly normal, indications are that calcium might have been a limiting factor with the smaller lime treatments.

In regard to nitrogen, Karraker (9) noted that nodulation was correlated with soil reaction fully as well when a particular reaction affected only a part of the roots of a plant as when it affected the entire root system.

According to this, if the reaction is proper in one part of the soil, good nodulation will take place. This experiment shows that in the limed layers good nodulation took place. Although, no doubt, good nodulation influenced the yield, it did not cause or prevent root penetration. There was only an occasional nodule found below 6 inches in any of the tile; yet, where conditions were favorable, root penetration was below this depth.

#### *Aluminum toxicity*

Aluminum toxicity has been advanced by Blair and Prince (1), Skeen (15), McLean and Gilbert (11), and others as one of the controlling factors in limiting plant growth at low pH values.

Magistad (12) presents water culture data to show that at acidities greater than a pH of 5.0 all the crops tried, except alfalfa, suffered both strong aluminum toxicity and strong H-ion toxicity; however, alfalfa died whether aluminum was added or not, showing that a pH of under 5.0 is definitely detrimental to alfalfa. His results also show no aluminum toxicity toward alfalfa with pH values above 5.0. Although it is almost impossible to differentiate between aluminum toxicity and hydrogen-ion toxicity, this work of Magistad would indicate that aluminum is not necessarily the limiting factor with the experiment reported here.

*High hydrogen-ion concentration*

As just stated, it is difficult to separate aluminum and hydrogen-ion toxicity or the subsequent resulting effects from their ionic concentration; however, it is thought that the lack of penetration of the roots in this experiment was due primarily to hydrogen-ion concentration.

Joffe (8) showed that the germination of alfalfa seed was practically the same with pH values of soil varying from 4.5 to 7.0 but was greatly reduced in cultures which yielded soil extracts having pH values below 4.5. Yields of alfalfa tops showed a gradual increase with an increase in the pH values of soil extracts from 3.8 to 7.0. Bryan (3) showed that alfalfa will not establish itself at a pH of 4.0 and produced its maximum growth at a pH of 7.0 and 8.0. Sewell and Gainey (14) conclude that the reaction of the soil, if within a range of pH 4.5 to 7.0, is a minor factor affecting growth of alfalfa if all the needed nutrients are supplied. Magstad (12) found that alfalfa suffered greatly at a pH of 5.0.

Results of the tile experiment show decidedly that good root growth was not obtained in soil under the conditions of the experiment at a pH of 4.7.

In summarizing the cause of the behavior of the roots to the various soil reactions, it would seem that below a pH of 5.0 the hydrogen-ion concentration has a direct effect on the roots in inhibiting growth and therefore good penetration. Where the pH is raised above a pH of 5.0, no doubt other factors enter in to promote better root development. This is probably due to the increased amount of calcium for plant nutritional purposes and increased biological activities brought about by higher pH values. This is shown by the increased vigor of the plants and roots of the experiment as the pH approached more nearly the neutral point.

The results also show that surface liming, by its influence on the substrata, will increase root penetration. That surface application of lime will change the reaction of the substrata is now well established. Nelson (13) and Wilson (20) have shown this to be the case. Analyses made by the writer on virgin soil and limed soil throughout the state of Pennsylvania also have shown this to be true. The amount of change, however, will depend on the rate of application and the length of the liming practice.

The experiments reported in this paper show that the pH of the second 3 inches of soil immediately below the limed strata is materially raised, and, over a 2-year period with moderate liming, possibly the pH of the strata below this may be raised slightly. It is also seen that where such changes have occurred in the pH value in the sublayer root penetration is obtained.

**SUMMARY**

The experiment reported here was conducted with alfalfa grown on a Dekalb silt loam surface soil placed in large sewer tile. The surface and sublayers of the soil were treated with varying quantities of limestone.

The pH value of the surface soil varied with the season to some extent and

on the limestone treated tile was slightly lower on an average the second year than the first.

The pH and replaceable calcium by 0.1 *N* HCl extraction had a correlation of  $0.856 \pm 0.0165$  over the period of two seasons.

The pH values of the sublayers were influenced materially by the calcium carbonate additions to the layers above, the amount depending on both the quantity of limestone added to the upper layer and that added to the lower layer. The depth of effect on pH in the sublayer depended also to some extent on length of liming history.

Root development correlated definitely with the pH and with the replaceable calcium of the various soil layers. A pH of 4.8 checked root growth almost entirely; a pH slightly above this, retarded root growth. The roots grew well in a pH above 5.

Depth of root penetration is definitely correlated with the yield of alfalfa, under the conditions of this experiment.

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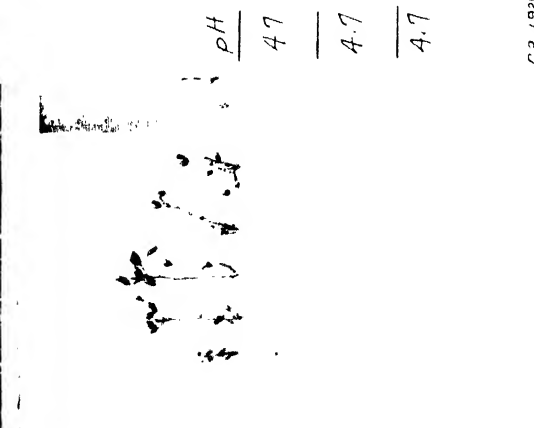
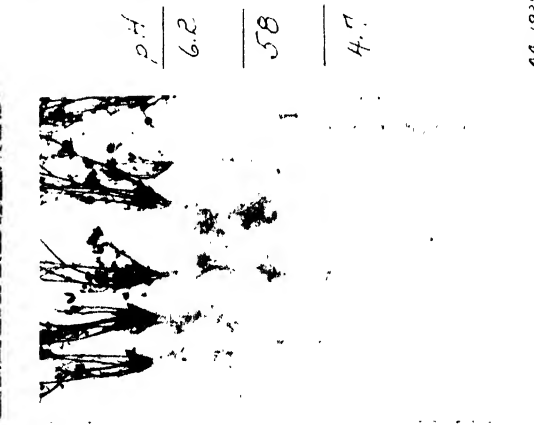
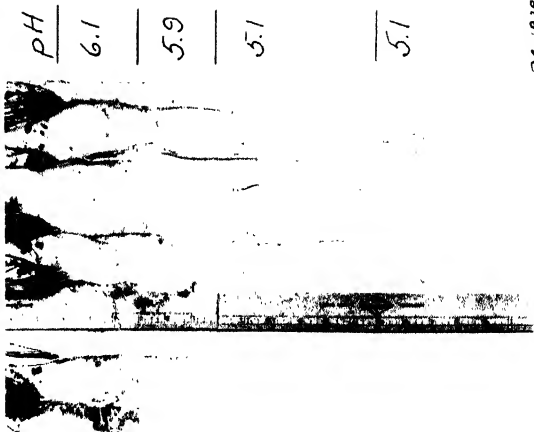
### PLATE 1

#### EFFECT OF DIFFERENT LIMESTONE TREATMENTS OF SOIL ON GROWTH AND ROOT DEVELOPMENT OF ALFALFA

FIG. 1. No limestone.

FIG. 2. Surface 3 inches, full lime requirement; sublayer, no lime.

FIG. 3. Surface 3 inches, full lime requirement; sublayer, one-fourth lime requirement.



134, 1928

44, 1928

62, 1928

FIG. 3

FIG. 2

FIG. 1

## PLATE 2

EFFECT OF DIFFERENT LIMESTONE TREATMENTS OF SOIL ON GROWTH AND  
ROOT DEVELOPMENT OF ALFALFA

FIG. 1. Surface 3 inches, full lime requirement; sublayer, one-half lime requirement

FIG. 2. Surface 3 inches, one-half lime requirement; sublayer, no lime.

FIG. 3. Surface 3 inches, one-half lime requirement; sublayer, one-fourth lime requirement.



FIG. 1

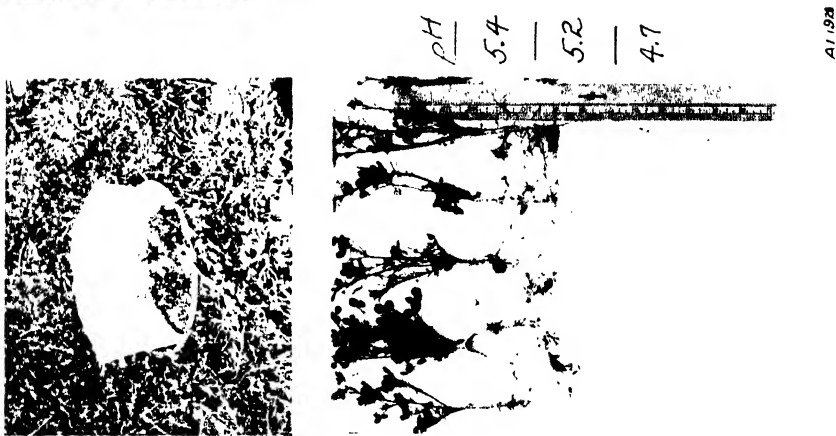


FIG. 2



FIG. 3

## PLATE 3

EFFECT OF DIFFERENT LIMESTONE TREATMENTS OF SOIL ON GROWTH AND  
ROOT DEVELOPMENT OF ATTALEA

FIG. 1. One half lime requirement.

FIG. 2. Surface 6 inches, one half lime requirement; sublayer, no lime



pH  
5.5  
—  
5.6  
—  
5.5  
—  
5.4

C1, 1928

FIG. 1



pH  
5.5  
—  
5.6  
—  
5.0

D2, 1928

FIG. 2



# NUTRITION STUDIES WITH CORN: I. A STATISTICAL INTERPRETATION OF THE NUTRIENT ION EFFECT UPON GROWTH IN ARTIFICIAL CULTURE<sup>1</sup>

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The purpose of these experiments is to test a somewhat different method of approach in the interpretation of physiological balance studies in relation to plant growth.

Since the adoption by Tottingham (9) of a system of graphical representation in the study of physiological balance, the idea of controlled variation of plant nutrients in the growth medium has been further developed and utilized in various ways. The historical background prior to 1914 is thoroughly summarized by Tottingham in a paper presenting the four-salt, pyramidal system for nutritional study (9). In 1915, Shive presented his triangular system, combining into three-salt solutions (8) the six major ions most commonly used in nutritional work. Jones and Shive (5) modified Tottingham's four-salt solution, replacing the  $\text{KNO}_3$  with  $(\text{NH}_4)_2\text{SO}_4$ , for purposes of comparing the influence of the nitrate ion,  $\text{NO}_3^-$ , with that of the ammonium ion,  $\text{NH}_4^+$ .

Livingston and Tottingham (7), in 1918, numbered and catalogued the six possible three-salt solutions which can supply the six major ions. Johnston (4), Gericke (3), and others, by working with these six types of solutions, were able to bring out some of the metabolic relationships of the various ions by comparisons among the types.

The simplicity of the three-salt solution makes it a valuable tool for many types of research, but any such solutions have the obvious disadvantage that if the proportion of one ion of a salt is altered, the accompanying ion of the salt must be varied in like proportion. For this reason, a system in which the relative concentration of each ion could be varied separately should greatly simplify the interpretation of the physiological significance of that ion.

## CALCULATION OF THE SOLUTIONS

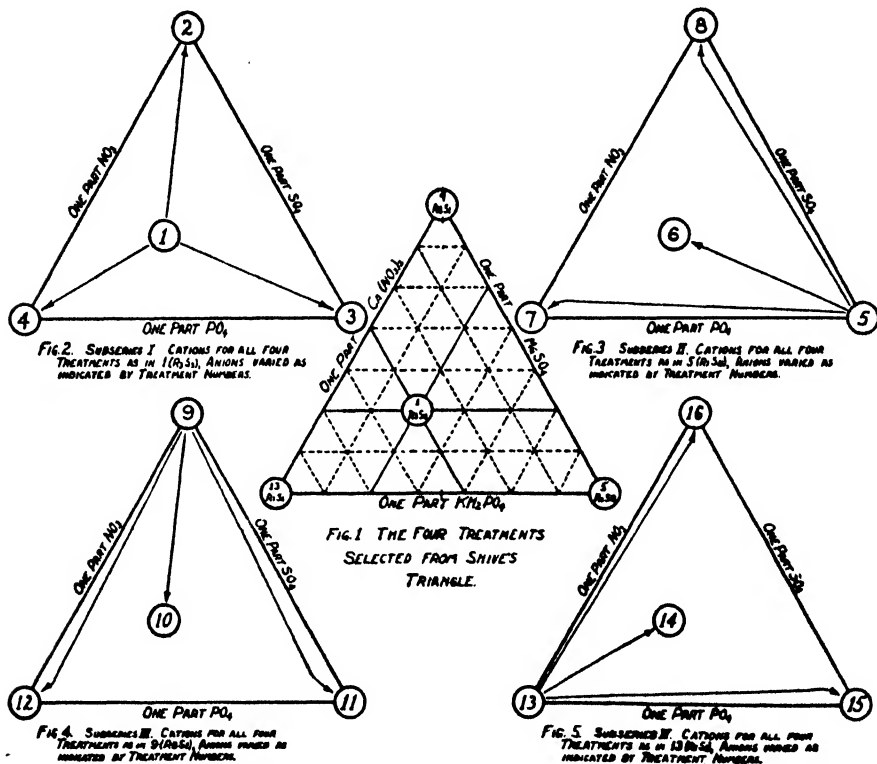
In order to create a system in which the concentration of any one ion may be varied as desired with that of any other ion, all nine of the salts used by Livingston and Tottingham are needed. This would require a vastly more intricate

<sup>1</sup> Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.



system, provided that some other means of simplification were not introduced. In order to effect this simplification, it was decided to use each ion at only three concentrations, i.e., corresponding to three multiples of the one-tenth increment of the total concentration.

The solutions were devised directly from the triangle of Shive (8), using the solutions represented by the three apexes and one centrally located point,  $R_3C_3$ , as bases for computations and comparisons. Figure 1 is a reproduction of Shive's triangle, with the four numbered solutions of this series indicating



FIGS. 1-5. DIAGRAMS REPRESENTING THE DERIVATION OF THE SOLUTIONS FOR SUBSERIES 1 TO 4 FROM SHIVE'S TRIANGLE

the focal points selected. From each of these focal points, the concentration of anions was varied with each of the other points, with the cations held constant. For example, there were four distinct solutions employed in which the cations were maintained in the same proportion as in Shive's  $R_3C_3$ , but the anions were present in four different proportions which correspond to Shive's  $R_3C_1$ ,  $R_1C_3$ ,  $R_1C_1$ , and  $R_3C_3$ . This manipulation is indicated in figure 2. Similarly, figure 3 represents the four solutions in which the cations were supplied in the ratio of  $R_1C_3$  and the anions varied as previously mentioned. Solutions in which the cation ratios were maintained as in  $R_3C_1$  and in  $R_1C_1$  are indicated

by figures 4 and 5, respectively. This manipulation of the anions and cations separately was accomplished by the use of nine salts, as already mentioned. Such an arrangement gives four subseries of four treatments each, as is indicated in the diagrams. A similar arrangement, wherein the cation concentration is varied to each of the focal points while the anion concentration is held constant, gives four further subseries, but these, which will be considered as subseries 5-8, are merely rearrangements of the same 16 treatments.

It is impossible to control absolutely either the osmotic proportions or the chemically equivalent proportions of the ions used in varying the ionic concentrations for the various solutions. Valence differences of the ions and differences in the ionization constants of the salts do not, however, prevent close approximations of the calculated ionic proportions.

In preparing these solutions, the procedure has been first to ascertain the multiples of the osmotic increment (one-tenth in this case) and the corresponding partial volume-molecular concentrations of the three salts which would be present in a solution of the stipulated cation proportion. Thus, for a solution with cations in the  $R_3C_3$  ratio at 0.5 atmosphere concentration, these would be:

<i>Salt</i>	<i>No. of increments</i>	<i>Volume molecular</i>
$KH_2PO_4$ .....	3	.0031
$Ca(NO_3)_2$ .....	3	.00225
$MgSO_4$ .....	4	.0057

Next, the multiples of the osmotic increment and the corresponding partial volume-molecular concentrations of the three salts which would be present in a solution of a given anion proportion are considered. If the anions should be in the same proportions as in  $R_8S_1$  (0.5 atmos. conc.), these would be:

<i>Salt</i>	<i>No. of increments</i>	<i>Volume molecular</i>
$KH_2PO_4$ .....	8	.0082
$Ca(NO_3)_2$ .....	1	.00075
$MgSO_4$ .....	1	.0014

In combining these ratios, one for the cations and the other for the anions, it at once becomes apparent that the three salts considered would supply 5 parts too few of  $H_2PO_4^-$ , 2 parts too many of  $NO_3^-$ , and 3 parts too many of  $SO_4^{--}$  when the desired cation relationships were attained. To obviate this, some of the  $Ca^{++}$  and  $Mg^{++}$  must be supplied as  $H_2PO_4^-$ . A solution of the following composition would, therefore, contain cations as in  $R_3C_3$  and anions as in  $R_8C_1$ :

<i>Salt</i>	<i>No. of increments</i>	<i>Volume molecular</i>
$KH_2PO_4$ .....	3	.0031
$Ca(NO_3)_2$ .....	1	.00075
$MgSO_4$ .....	1	.0014
$CaH_4(PO_4)_2$ .....	2	.0015
$MgH_4(PO_4)_2$ .....	3	.0043

It should be emphasized, however, that the part of the total concentration due to cations is divided into tenths, as are the salts of Shive's triangle, and that

TABLE 1

*Partial volume-molecular concentrations of salts in culture solutions at total concentrations of approximately 0.5 atmosphere possible osmotic pressure*

SOLUTION NUMBERS	KH <sub>2</sub> PO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	Ca (H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	Mg (H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	KNO <sub>3</sub>	Mg(NO <sub>3</sub> ) <sub>2</sub>	K <sub>2</sub> SO <sub>4</sub>	CaSO <sub>4</sub>
1. R <sub>3</sub> C <sub>3</sub>	.0031	.00225	.0057						
Cations as in R <sub>3</sub> C <sub>3</sub>									
2. Anions as in R <sub>3</sub> C <sub>1</sub>	.0031	.00075	.0014	.0015	.0043				
Cations as in R <sub>3</sub> C <sub>3</sub>									
3. Anions as in R <sub>1</sub> C <sub>3</sub>	.0010	.00225	.0014			.0021	.0043		
Cations as in R <sub>3</sub> C <sub>3</sub>									
4. Anions as in R <sub>1</sub> C <sub>1</sub>	.0010	.00075	.0057					.0021	.0015
Cations as in R <sub>1</sub> C <sub>3</sub>									
5. Anions as in R <sub>1</sub> C <sub>3</sub>	.0010	.0060	.0014						
Cations as in R <sub>1</sub> C <sub>3</sub>									
6. Anions as in R <sub>3</sub> C <sub>3</sub>	.0010	.00225	.0014	.0015					.0015
Cations as in R <sub>1</sub> C <sub>3</sub>									
7. Anions as in R <sub>1</sub> C <sub>1</sub>	.0010	.00075	.0014						.00525
Cations as in R <sub>1</sub> C <sub>3</sub>									
8. Anions as in R <sub>3</sub> C <sub>1</sub>	.0010	.00075	.0014	.00525					
Cations as in R <sub>3</sub> C <sub>1</sub>									
9. Anions as in R <sub>3</sub> C <sub>1</sub>	.0082	.00075	.0014						
Cations as in R <sub>3</sub> C <sub>1</sub>									
10. Anions as in R <sub>3</sub> C <sub>3</sub>	.0031	.00075	.0014			.0021		.0021	
Cations as in R <sub>3</sub> C <sub>1</sub>									
11. Anions as in R <sub>1</sub> C <sub>3</sub>	.0010	.00075	.0014			.0072			
Cations as in R <sub>3</sub> C <sub>1</sub>									
12. Anions as in R <sub>1</sub> C <sub>1</sub>	.0010	.00075	.0014					.0072	
Cations as in R <sub>1</sub> C <sub>1</sub>									
13. Anions as in R <sub>1</sub> C <sub>1</sub>	.0010	.00075	.0112						
Cations as in R <sub>1</sub> C <sub>1</sub>									
14. Anions as in R <sub>3</sub> C <sub>3</sub>	.0010	.00075	.0042		.0043		.0043		
Cations as in R <sub>1</sub> C <sub>1</sub>									
15. Anions as in R <sub>1</sub> C <sub>3</sub>	.0010	.00075	.0014				.0098		
Cations as in R <sub>1</sub> C <sub>1</sub>									
16. Anions as in R <sub>3</sub> C <sub>1</sub>	.0010	.00075	.0014		.0098				

any one cation may be varied in relative concentration with the other two, as long as the total number of increments remains at ten. If it is decided that eight parts of the cation concentration is to be due to  $K^+$ , then the parts of both  $Mg^{++}$  and  $Ca^{++}$  must be one each; and if it is desired to utilize eight parts of the total concentration of the anions as  $NO_3^-$ , then the parts of  $SO_4^{--}$  and  $PO_4^{--}$  must be one each. This solution is treatment 11 of this series, and the quantities of the various salts used are listed in tables 1 and 2. Table 1 gives the partial volume-molecular composition of the various treatments, with a total solution concentration of approximately 0.5 atmosphere. Table 2 gives the number of milliliters of stock solution required for each liter of nutrient

TABLE 2

*Stock solutions required to produce 1 liter of nutrient solution, with total osmotic concentration of 0.5 atmosphere*

TREATMENT NUMBER	0.5 M $KH_2PO_4$	0.5 M $Ca(NO_3)_2$	0.5 M $MgSO_4$	0.05 M $CaH_4$ ( $PO_4$ ) <sub>2</sub>	0.05 M $MgH_4$ ( $PO_4$ ) <sub>2</sub>	0.5 M $KNO_3$	0.5 M $Mg$ ( $NO_3$ ) <sub>2</sub>	0.5 M $K_2SO_4$	0.01 M $CaSO_4$
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
1	6 2	4 5	11.4						
2	6.2	1 5	2 8	30 0	86 0				
3	2 0	4.5	2 8			4 2	8 6		
4	2.0	1.5	11.4					4.2	150.0
5	2 0	12 0	2 8						
6	2 0	4 5	2 8	30 0					150.0
7	2.0	1.5	2.8						525 0
8	2.0	1.5	2 8	105.0					
9	16 4	1.5	2.8						
10	6 2	1.5	2.8			4 2		4 2	
11	2 0	1 5	2.8			14.4			
12	2.0	1.5	2.8					14.4	
13	2.0	1.5	22.4						
14	2 0	1.5	8.4		86.0		8.6		
15	2.0	1 5	2.8				19.6		
16	2.0	1.5	2.8		196.0				

solution. The minor elements, iron, boron, and manganese were added to all solutions at the rate of 0.5 p.p.m.

Finally, the literature dealing with culture solutions contains statements that the two ions of any salt may retain a certain affiliation with each other when in solution, an assumption which has been based on measurements of absorption rates. If such were indeed the case, this revised system of physiological balance is obviously unjustifiable. Comparatively recent physical chemical research, however, justifies the assumption that mixtures of ions in solution have no such bonds of affiliation. The Debye-Milner theory of strong electrolytes (2) points out that electrical bonds between ions in solution do

exist, and that each ion in the solution has its electrical potential which exerts an influence over a limited sphere. However, inter-ionic forces are not specific in respect to the attraction of any negatively charged ion to a particular positively charged ion. And since X-ray analyses of crystal structure of these electrolytes have convinced physical chemists that the ions of strong electrolytes are actually in the ionic state even in the crystal, then in solution surely no such specific bonds develop. The greater burden of evidence substantiates this view. For a complete discussion of the physical properties of ions of strong electrolytes, see *Electrolytes* by Falkenhagen (2).

#### APPLICATION OF THE EXPERIMENTAL SYSTEM

Seed of the 1934 crop of Dave Croshaw's strain of Reed's yellow dent corn was furnished for the experiment by Dr. H. B. Sprague of the Agronomy Department of the New Jersey Agricultural Experiment Station. Only seeds selected for uniformity in size, weight, and shape of kernel were used. These were sown on April 20, 1935, in washed, white quartz sand, in 3-gallon glazed earthenware percolators. Twelve seeds, uniformly distributed, were sown in each percolator. Four uniform seedlings were selected from each percolator, and the others were removed by flooding the percolator with water and withdrawing the plants. In this way, the entire root system is easily removed, so that a minimum of organic material is left in the sand.

Sixty percolators of seedlings were started, and the forty-eight most uniform were selected for the experiment. This permitted three replications of each of the sixteen treatments. Solutions were first applied 9 days from planting and continued for 40 days, when the plants were harvested. Tap water was used to germinate the seeds.

Each culture received 2 liters of solution a day, one applied by the constant drip method, and the other in one application in the morning to flush the old solution from the sand.

During the course of growth, notes on differences were taken as they appeared. The plants were photographed 2 weeks before harvest, at which time the treatments high in  $Mg^{++}$  were showing rather severe toxicity symptoms. The younger leaves were tightly curled, and the edges of the blades were cracked and distorted. At the time of harvest, however, the plants had partially recovered from this injury without recourse to a change in treatment.

At the harvest, the plants were cut off just above the first visible node. Total green weight per plant was determined, and the leaves and stems were separated for analysis. The stem fraction included the stem, the leaf sheaths, and all tissue below the uppermost visible junction between leaf and leaf sheath.

These fractions of all the plants from each treatment were then diced and thoroughly mixed, and aliquots were weighed out for the various determinations. Dry weights were determined on duplicate aliquots. The samples were dried to constant weight at a temperature of 70–75°C.

## PRESENTATION OF DATA

With such large differences between the solutions applied, it is to be expected that growth variations would be relatively extreme. Plates 1 and 2 give a visual conception of growth differences, which are presented in terms of green weight per plant in tables 3 and 4. The plants are arranged in subseries order, according to the ion present in high concentration. By inspection, the gross effect of the relative concentrations of the other ions present may be compared.

The data from the dry weight determinations are included in tables 5 and 6.

TABLE 3

*Yields from the various treatments, arranged in subseries for comparison of anion effect upon growth*

SUB-SERIES NUMBER	TREATMENT NUMBER	CATION PROPORTIONS			ANION PROPORTIONS			RANK	AVERAGE FRESH WEIGHT PER PLANT	AVERAGE DRY WEIGHT PER PLANT
		Ca <sup>++</sup>	Mg <sup>++</sup>	K <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	PO <sub>4</sub> <sup>-</sup>			
1	1	3	4	3	3	4	3	**	gm. 310 ± 11.8	gm. 26.36
	2	3	4	3	1	1	8		172 ± 7.6	15.76
	3	3	4	3	8	1	1	***	407 ± 24.5	34.60
	4	3	4	3	1	8	1	*	175 ± 3.6	15.95
2	5	8	1	1	8	1	1	***	297 ± 15.2	26.45
	6	8	1	1	3	4	3	**	269 ± 4.2	26.08
	7	8	1	1	1	8	1	*	161 ± 2.3	17.70
	8	8	1	1	1	1	8		130 ± 4.7	14.11
3	9	1	1	8	1	1	8		150 ± 2.9	15.02
	10	1	1	8	3	4	3	**	277 ± 14.7	24.20
	11	1	1	8	8	1	1	***	428 ± 14.1	32.59
	12	1	1	8	1	8	1	*	159 ± 2.9	15.88
4	13	1	8	1	1	8	1		112 ± 7.3	11.74
	14	1	8	1	3	4	3	**	158 ± 12.1	12.39
	15	1	8	1	8	1	1	***	173 ± 5.9	16.70
	16	1	8	1	1	1	8	*	129 ± 8.0	13.85

\*\*\* Signifies highest yielding treatment in subseries.

\*\* Signifies 2nd highest yielding treatment in subseries.

\* Signifies 3rd highest yielding treatment in subseries.

The per cent dry weight per plant is a calculated value, inasmuch as the actual per cent dry weight determinations were based on the stem and leaf fractions of the tissue. Individual weighings of the fresh leaf and stem fractions were taken at harvest, and the dry weight per plant was calculated from this and from the per cent dry weight of leaves and stems.

## ANALYSIS OF DATA AND DISCUSSION OF RESULTS

The solutions in each of the subseries 1 to 4 are characterized by fixed cation proportions. Thus, in table 3, the different anion proportions for each sub-

series may be compared as they affect the yield in grams of green weight. It will be noted that for each of the four fixed cation proportions, the treatments high in  $\text{NO}_3^-$  and low in  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  gave greater yields than did any other treatment. The anion proportions referred to  $\text{R}_3\text{C}_3$  ranked second in each case, and the 1-8-1 proportions, high in  $\text{SO}_4^{2-}$ , ranked third, except in subseries 4. The differences between the high  $\text{SO}_4^{2-}$  and high  $\text{PO}_4^{3-}$  treatments are negligible in comparison to the other differences. The differences in yield associated with variations in anion proportions for given cation propor-

TABLE 4  
*Yields from the various treatments, arranged in subseries for comparison of cation effect upon growth*

SUB-SERIES NUMBER	TREATMENT NUMBER	CATION PROPORTIONS			ANION PROPORTIONS			RANK	AVERAGE FRESH WEIGHT PER PLANT	AVERAGE DRY WEIGHT PER PLANT
		$\text{Ca}^{++}$	$\text{Mg}^{++}$	$\text{K}^+$	$\text{NO}_3^-$	$\text{SO}_4^{2-}$	$\text{PO}_4^{3-}$			
									gm.	gm.
5	1	3	4	3	3	4	3	***	$310 \pm 11.8$	26.36
	6	8	1	1	3	4	3	*	$269 \pm 4.2$	26.06
	10	1	1	8	3	4	3	**	$277 \pm 14.7$	24.20
	14	1	8	1	3	4	3		$158 \pm 12.1$	12.39
6	2	3	4	3	1	1	8	***	$172 \pm 7.6$	15.76
	8	8	1	1	1	1	8	*	$130 \pm 4.7$	14.11
	9	1	1	8	1	1	8	**	$150 \pm 2.9$	15.02
	16	1	8	1	1	1	8		$129 \pm 8.0$	13.85
7	3	3	4	3	8	1	1	**	$407 \pm 24.5$	34.60
	5	8	1	1	8	1	1	*	$297 \pm 15.2$	26.45
	11	1	1	8	8	1	1	***	$428 \pm 14.1$	32.59
	15	1	8	1	8	1	1		$173 \pm 5.9$	16.70
8	4	3	4	3	1	8	1	***	$175 \pm 3.6$	15.95
	7	8	1	1	1	8	1	**	$161 \pm 2.3$	17.70
	12	1	1	8	1	8	1	*	$159 \pm 2.9$	15.88
	13	1	8	1	1	8	1		$112 \pm 7.3$	11.74

\*\*\* Signifies highest yielding treatment in subseries.

\*\* Signifies 2nd highest yielding treatment in subseries.

\* Signifies 3rd highest yielding treatment in subseries.

tions were greatest when the cation proportions were high in  $\text{K}^+$ , and least when they were high in  $\text{Mg}^{++}$ .

Hence, such comparisons show that the relative  $\text{NO}_3^-$  concentration is the limiting anion for the growth of corn under the conditions of this experiment, a conclusion previously reached by several workers; and also, that effectiveness of nitrate variation in producing changes in growth depends upon the attending proportion of the positively charged ions. It is evident that the  $\text{NO}_3^-$  is limiting even in the  $\text{R}_3\text{C}_3$  concentration, and that this very pronounced importance of the  $\text{NO}_3^-$  completely overshadows the effect of the other two anions.

TABLE 5  
Yield data for plants grown in culture solutions, grouped to determine the effect of anions upon growth

SUB-SERIES NUMBER	TREATMENT NUMBER	CATION PROPORTIONS	ANION PROPORTIONS	AVERAGE FRESH WEIGHT PER PLANT	AVERAGE DRY WEIGHT PER PLANT	PER CENT DRY WEIGHT PER PLANT	PER CENT DRY WEIGHT STEMS	PER CENT DRY WEIGHT LEAVES	WATER PER GRAM DRY WEIGHT STEMS	WATER PER GRAM DRY WEIGHT LEAVES
				gm.	gm.				gm.	gm.
1	1	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>3</sub> C <sub>3</sub>	307.7	26.36	8.56	6.47	14.31	14.45	5.99
	2	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>8</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>3-</sup> )	171.2	15.76	9.20	6.28	14.38	14.92	5.92
	3	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	407.3	34.60	8.49	5.76	14.03	16.48	6.12
	4	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>2-</sup> )	175.1	15.95	9.11	6.28	14.59	14.92	5.86
2	5	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	295.6	26.45	8.95	5.76	14.70	16.36	5.80
	6	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>3</sub> C <sub>3</sub>	260.6	26.08	10.00	6.92	15.47	13.45	5.46
	7	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>2-</sup> )	161.3	17.70	10.96	7.72	16.91	11.96	4.92
	8	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>8</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>3-</sup> )	130.4	14.11	10.81	7.51	16.37	12.31	5.11
3	9	As in R <sub>8</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>8</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>3-</sup> )	149.5	15.02	10.04	6.64	14.65	14.06	5.82
	10	As in R <sub>8</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>3</sub> C <sub>3</sub>	275.1	24.20	8.80	6.08	14.35	15.45	5.97
	11	As in R <sub>8</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	427.3	32.59	7.62	5.09	13.14	18.64	6.61
	12	As in R <sub>8</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>2-</sup> )	158.7	15.88	10.00	6.98	15.44	13.33	5.48
4	13	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>2-</sup> )	112.5	11.74	10.44	7.53	15.27	12.28	5.54
	14	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>3</sub> C <sub>3</sub>	146.7	12.39	8.45	5.71	12.96	16.51	6.72
	15	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	164.3	16.70	10.15	6.18	13.76	15.18	6.26
	16	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>8</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>3-</sup> )	129.1	13.85	10.75	7.68	15.95	12.02	5.27



TABLE 6  
Yield data for plants grown in culture solutions, grouped to determine the effect of cations on growth

SUB-SERIES NUMBER	TREATMENT NUMBER	CATION PROPORTIONS	ANION PROPORTIONS	AVERAGE FRESH WEIGHT PER PLANT	AVERAGE DRY WEIGHT PER PLANT	PER CENT DRY WEIGHT PER PLANT	PER CENT DRY WEIGHT STEMS	PER CENT DRY WEIGHT LEAVES	WATER PER GRAM DRY WEIGHT STEMS	WATER PER GRAM DRY WEIGHT LEAVES
				gm.	gm.				gm.	gm.
5	1	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>3</sub> C <sub>3</sub>	307.7	26.36	8.58	6.47	14.31	14.45	5.99
	6	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>3</sub> C <sub>3</sub>	260.6	26.08	10.00	6.92	15.47	13.45	5.46
	10	As in R <sub>9</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>3</sub> C <sub>3</sub>	275.1	24.20	8.80	6.08	14.35	15.45	5.97
	14	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>3</sub> C <sub>3</sub>	146.7	12.39	8.45	5.71	12.96	16.51	6.72
6	2	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>9</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>=</sup> )	171.2	15.76	9.20	6.28	14.38	14.92	5.92
	8	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>9</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>=</sup> )	130.4	14.11	10.81	7.51	16.37	12.31	5.11
	9	As in R <sub>8</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>9</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>=</sup> )	149.5	15.02	10.04	6.64	14.65	14.06	5.82
	16	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>9</sub> C <sub>1</sub> (high PO <sub>4</sub> <sup>=</sup> )	129.1	13.85	10.73	7.68	15.95	12.02	5.27
7	3	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	407.3	34.60	8.49	5.76	14.03	16.48	6.12
	5	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	295.6	26.45	8.95	5.76	14.70	16.36	5.80
	11	As in R <sub>9</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	427.3	32.59	7.62	5.09	13.14	18.64	6.61
	15	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>1</sub> C <sub>8</sub> (high NO <sub>3</sub> <sup>-</sup> )	164.3	16.70	10.15	6.18	13.76	15.18	6.26
8	4	As in R <sub>3</sub> C <sub>3</sub>	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>-</sup> )	175.1	15.95	9.11	6.28	14.59	14.92	5.86
	7	As in R <sub>1</sub> C <sub>8</sub> (high Ca <sup>++</sup> )	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>-</sup> )	161.3	17.70	10.96	7.72	16.91	11.96	4.92
	12	As in R <sub>9</sub> C <sub>1</sub> (high K <sup>+</sup> )	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>-</sup> )	158.7	15.88	10.00	6.98	15.44	13.33	5.48
	13	As in R <sub>1</sub> C <sub>1</sub> (high Mg <sup>++</sup> )	As in R <sub>1</sub> C <sub>1</sub> (high SO <sub>4</sub> <sup>-</sup> )	112.5	11.74	10.44	7.53	15.27	12.28	5.54

The arrangement of treatments in table 4 allows for a similar inspection of the data on the cations. Thus, the  $R_3C_3$  ratio of cation distribution shows the highest yield in all but subseries 7, in which the mean deviation in yields statistically nullifies the significance. The comparisons here are a bit irregular, but in general, high  $K^+$  concentration is associated with slightly higher yields than is high  $Ca^{++}$ , which in turn is definitely superior to high  $Mg^{++}$  concentration. It is evident that cation variations are associated with the most pronounced growth variations when the anion distribution shows a high proportion of nitrate; and, conversely, cation variations are associated with least growth variation when nitrate supply is low and either phosphate or sulfate high. Of the cations, then, it appears that relatively larger proportions of  $K^+$  and  $Ca^{++}$  are required than of  $Mg^{++}$ , in order to approach optimal growth.

Such an analysis does not attempt to show the actual relative contribution of each ion to the growth of the plant. It must be remembered, also, that these growth differences found may be due to deficiency or toxicity effects, and neither of these are clarified in this type of analysis. In all probability, furthermore, cation relationships are further complicated by antagonistic effects. The statistical analysis should allow for these factors.

#### STATISTICAL CONSIDERATION OF DATA

Justification for a statistical analysis lies in two facts: first, that each ion was applied in three definite relative proportions, one, three, and eight out of the total parts due to the ions carrying the common positive or negative charges; and, secondly, that each of these proportions was tested against a large number of individual plants.

In order to study the effect of the independent variables (the concentrations of the various ions) upon the dependent variable (growth as measured by grams green weight per plant), a multiple correlation analysis was employed. The fact that a purely mathematical analysis, in which the constants are determined by the method of least squares, entails such burdensome computations, led to the selection of the method of successive approximations as applied to curvilinear relationships (1). The regression equation upon which the computations were based is as follows:

$$X_1 = a' + f(X_2) + f(X_3) + f(X_4) + f(X_5) + f(X_6) + f(X_7)$$

In this equation, the dependent variable,  $X_1$ , is the average green weight of the plant; and of the independent variables,  $X_2$  is the  $NO_3^-$ ,  $X_3$  the  $K^+$ ,  $X_4$  the  $PO_4^{=}$ ,  $X_5$  the  $Ca^{++}$ ,  $X_6$  the  $SO_4^{=}$ , and  $X_7$  the  $Mg^{++}$  concentration. The factor  $a'$  represents the effect on growth of all the factors, both genetic and environmental, which were held constant according to the conditions of the experiment. Briefly, this equation is a very general statement, in mathematical terms, that the growth of any selected plant is due to the influence of all environmental factors, which were the same for all plants, minimized genetic

differences, and some function of the influence of each of the ions which were varied in definite proportions, according to the conditions of the experiment.

The  $X'$  and  $Z'$  values, from which the part correlation indexes were determined, are presented in table 7. The  $X'$  terms represent the total statistical significance of the ion designated, in relation to growth in the various treatments. The  $Z'$  values are the unaccounted for residuals from the statistical analysis. The values plotted on the curves presented in figure 6 are derived from the  $X'$  values in the table. The curve that gives the yield corrected for  $\text{NO}_3^-$  represents the actual yield less that part of the yield due to the influence of the other five major ions; in other words, the constant  $a'$  is included in this curve. The zero abscissa of each of the other curves represents the  $\text{NO}_3^-$  curve

TABLE 7  
*Allowances and residuals from the statistical analysis*

TREAT- MENT NUMBER	$X_1$	$\text{NO}_3^-$		$\text{K}^+$		$\text{PO}_4^{=}$		$\text{Ca}^{++}$		$\text{SO}_4^{=}$		$\text{Mg}^{++}$	
		$x'_2$	$z'_2$	$x'_4$	$z'_4$	$x'_4$	$z'_4$	$x'_6$	$z'_6$	$x'_8$	$z'_8$	$x'_{10}$	$z'_{10}$
1	307.7	253	10	47	10	1	10	-1	10	0	10	0	10
2	171.2	150	-20	47	-20	-3	-20	-1	-20	-1	-20	0	-20
3	407.3	326	34	47	34	2	34	-1	34	-1	34	0	34
4	175.1	150	-24	47	-24	2	-24	-1	-24	1	-24	0	-24
5	295.6	326	-33	-45	-29	2	-29	30	-24	-1	-24	8	-23
6	260.6	253	12	-45	16	1	16	30	21	0	21	8	22
7	161.3	150	5	-45	9	2	9	30	14	1	14	8	15
8	130.4	150	-19	-45	-15	-3	-15	30	-10	-1	-10	8	-9
9	149.5	150	-19	43	-28	-3	-28	-16	-30	-1	-30	8	-29
10	275.1	253	-2	43	-11	1	-11	-16	-13	0	-13	8	-12
11	427.3	326	76	43	67	2	67	-16	65	-1	65	8	66
12	158.7	150	-19	43	-28	2	-28	-16	-30	1	-30	8	-29
13	112.5	150	36	-45	40	2	40	-16	38	1	38	-16	36
14	146.7	253	-19	-45	-15	1	-15	-16	-17	0	-17	-16	-19
15	164.3	326	-77	-45	-73	2	-73	-16	-75	-1	-75	-16	-77
16	129.1	150	60	-45	64	-3	64	-16	62	-1	62	-16	60

straightened out so that any point on the curve can be represented as a zero value. The corrections for the other ions on total yield are either positive or negative with respect to this abscissa.

It must be remembered, in regard to these curves, that the total anion concentration is limited to ten relative parts. Therefore, if the hypothetical yield of any treatment is to be derived from the  $\text{NO}_3^-$  curve, the sum of the anion concentrations of the  $\text{SO}_4^{=}$  and  $\text{PO}_4^{=}$  ions, plus the concentration of the  $\text{NO}_3^-$  ion at the point selected upon the upper curve, must total ten parts. Thus, if it is desired to compute the yield of a treatment with eight parts of  $\text{NO}_3^-$ , the correction on the  $\text{NO}_3^-$  curve for the effects of  $\text{PO}_4^{=}$  and  $\text{SO}_4^{=}$  must be taken from the point on the curves corrected for each of these ions represented by one relative part. Any cation distribution may be arbitrarily se-

lected, as long as the total comes to ten relative parts, and these corrections are then added to the  $\text{NO}_3^-$  curve at the point previously selected.

The indexes of part correlation were computed in the standard manner (1).

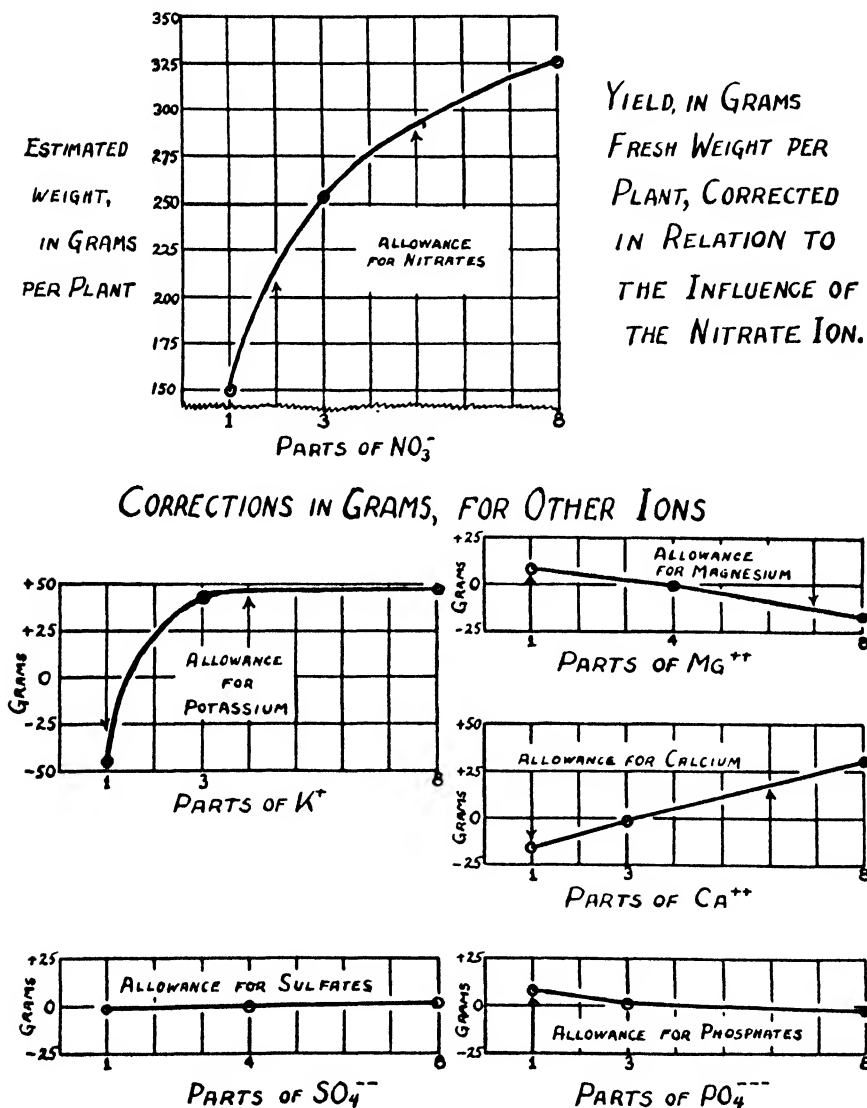


FIG. 6. NET REGRESSION CURVES FOR THE VARIOUS IONS

That is, if the net effect on growth due to a given ion is represented by  $D$ , and the difference between the observed yield and the summation of the net affects of all other ions except the one being considered is represented by  $\Delta$ ; then the coefficient of correlation between  $D$  and  $\Delta$  ( $r_{D\Delta}$ ) becomes the index of part cur-

vilinear correlation between growth and the given ion. These indexes as found for the various ions are presented in table 8.

These indexes, it should be recalled, show to what extent the variability indicated by the net regression curve of a given component accounts for the actual variability of this component. The indexes for  $\text{NO}_3^-$ ,  $\text{K}^+$ , and  $\text{Ca}^{++}$  are indicative of a pronounced correlation between the concentration of these ions and plant growth. The highest of the correlations occurs between the nitrate supply and growth, with an index of part correlation of 0.874, with the indexes for  $\text{K}^+$  and  $\text{Ca}^{++}$  0.774 and 0.471 respectively. This, of course, means that the net regression curve for nitrate in figure 6 comes very close to being a true picture of the relationship between nitrate supply and growth when the effect of all other factors has been eliminated, inasmuch as an index of 0.874 indicates that practically all of the variability has been taken into account. Correspondingly, the curve for the net effect of  $\text{K}^+$  concentration on growth represents the major portion of the growth variation associated with this ion. The curve for the net effect of calcium indicates the trend in growth variation accompanying variation in  $\text{Ca}^{++}$  concentration, but the index value of 0.471 shows that there are appreciable deviations which are not allowed for by this

TABLE 8  
*Indexes of part correlation*

	$\text{NO}_3^-$ 12-34567	$\text{PO}_4^{=}$ 14-23567	$\text{SO}_4^{=}$ 16-23457	$\text{K}^+$ 13-24567	$\text{Ca}^{+++}$ 15-23467	$\text{Mg}^{++}$ 17-23456
Index of part correlation.....	.874	none	none	.774	.471	.224

curve. The net regression curve for magnesium has a negative slope, suggesting the tendency of increasing  $\text{Mg}^{++}$  concentration to become deleterious. The index value of 0.224 for the part correlation between  $\text{Mg}^{++}$  supply and growth indicates that there was considerable variation not represented by the regression curve. With reference to table 7, the high variation in the residuals of the high magnesium treatments, from -77 to +60, at once explains why such a low correlation index is found. Such high residual values indicate that consideration of the effect of each ion separately will not satisfactorily account for the yield variations observed but that a joint relationship between all or some of the ions is probably involved. Unquestionably there must be a direct relationship between the  $\text{Mg}^{++}$  and  $\text{NO}_3^-$  ionic concentrations which this analysis does not bring out.

The fact that no correlation is observed in the case of the anions  $\text{SO}_4^{=}$  and  $\text{PO}_4^{=}$  with the growth of the plants, also evident in the horizontal net regression curves of figure 6, definitely establishes the fact that in no concentration used was there evidence of either deficiency or toxicity due to the concentrations of these ions. This fact simplifies a consideration of a possible joint functional relationship of growth to the relative concentrations of the four other ions; these four latter factors are, therefore, grouped together and con-

sidered as a joint factor influencing the growth of the plant. Since the  $\text{PO}_4^{=}$  and  $\text{SO}_4^{=}$  concentrations have no effect upon growth, within the limits of this experiment, they may be excluded from the study of this joint functional relationship. Figure 7 is a graphic representation of this relationship, and the modified regression equation becomes:

$$X_1 = a' + f(X_4) + f(X_6) + f[X_2, X_3, X_5, X_7]$$

In each of the three diagrams of figure 7, the growth at a definite  $\text{NO}_3^-$  supply is plotted against the relative cation concentration. The base of each prism represents the triangle as used by Shive, but with the anions omitted;

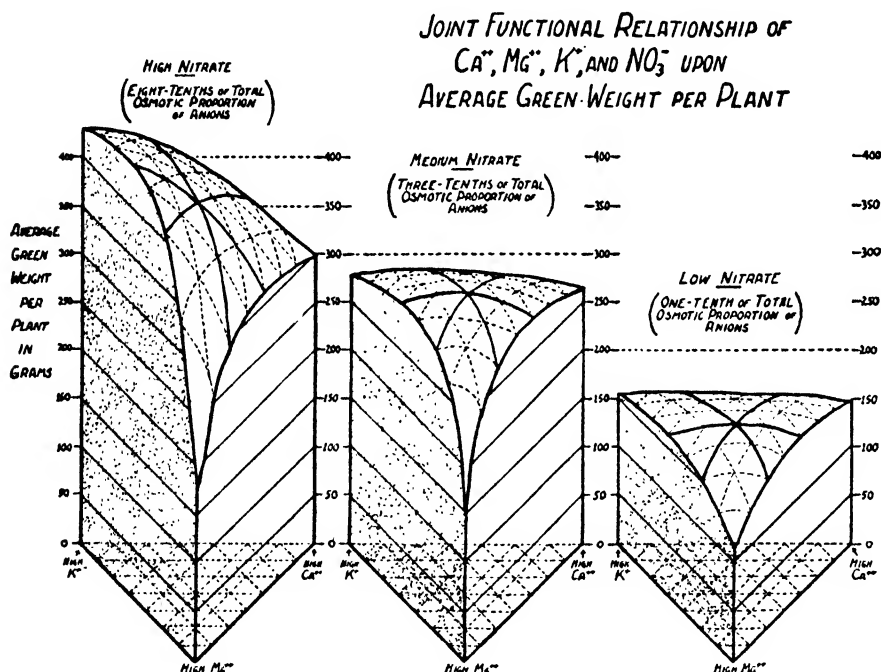


FIG. 7. INTERRELATIONSHIP OF THE CATIONS AND NITRATE SUPPLY IN THE SUBSTRATE, AS THEY INFLUENCE GROWTH OF THE PLANT

consequently it is representative of the cation distribution only. Each prism, therefore, represents the effect of the cation distribution upon growth, at a definite  $\text{NO}_3^-$  level. The  $\text{PO}_4^{=}$  and  $\text{SO}_4^{=}$  ions are completely omitted from consideration in this study, an omission justified by the facts that there was no calculable correlation between the relative concentrations of these ions and growth, and that the net regression curve for each of these ions was horizontal.

If in any of the prisms, therefore, there were no significant differences in terms of growth, due to the differences in the relative amounts of the cations supplied, the upper surface of that prism would be a duplication of the base. Also, if there were no correlation between the relative  $\text{NO}_3^-$  supply and the

relative cation distribution, the regression surface of all three prisms would be identical.

With this in mind, a comparison of the three prisms shows that the ability of the plant to utilize  $\text{NO}_3^-$  in increasingly high concentration is closely correlated with the cation distribution. The greater the distortion of the regularity of the triangular upper surface of the prism, the greater is the significance of the cation distribution. Therefore, if the  $\text{NO}_3^-$  is present in limited quantity, there is little significance in the cation distribution as this affects the growth of the plants. At the other extreme, with an abundance of  $\text{NO}_3^-$ , the ability of the plant to utilize this ion most efficiently is clearly limited by the relative cation distribution. In brief, then, the higher the supply of available  $\text{NO}_3^-$  in the substrate, the more important the relative cation distribution becomes.  $\text{K}^+$  would appear to be necessary in greater concentration than  $\text{Ca}^{++}$ , under conditions of high available  $\text{NO}_3^-$ , and  $\text{Mg}^{++}$  seems necessary in much lesser amounts than either  $\text{K}^+$  or  $\text{Ca}^{++}$ . On the other hand, the relatively flat upper regression surface of the prism which represents the plants grown at the low  $\text{NO}_3^-$  level suggests that here there can be little significance attached to the relative cation distribution. The toxicity symptoms observed in the high  $\text{Mg}^{++}$  treatments were correspondingly less significant at the low  $\text{NO}_3^-$  level.

This joint functional relationship suggests that a profitable means of study of cation balance in the substrate would be through a study of the nitrogen assimilation, as shown by the nitrogen fractions found in tissue analyses.

This type of consideration of the cations would indicate that cation ratios, as considered previously by other investigators, are not inclusive enough, and that any consideration of the cation supply must include the relative concentration of all three cations to have full significance. None of the three ratios,  $\text{Ca/Mg}$ ,  $\text{K/Mg}$ , or  $\text{K/Ca}$ , shows any significance in this series.

Table 7 includes the data of the dry weight determinations. It is evident here, too, that the nitrate supply is without question the factor which extends the greatest influence over the per cent of dry weight. In every treatment, both in the leaf and stem fractions, the number of grams of water per gram dry weight increases with increased  $\text{NO}_3^-$  in the nutrient medium. Probably stored carbohydrates in the low  $\text{NO}_3^-$  plants accounts for this in part, at least. This is in direct agreement with the bulk of accumulated data.

The treatments high in  $\text{K}^+$  in the nutrient solution all produced tissue higher in water content than those grown with high  $\text{Ca}^{++}$ . The necessity of soluble potassium at the rapidly growing regions is probably a factor here.

The treatments high in  $\text{Ca}^{++}$  were consistently higher in per cent dry weight than were any of the other treatments. Probably the factors which cause this condition are very complex, and may be demonstrable in the chemical or morphological analyses of the tissues.

The data from the high  $\text{Mg}^{++}$  treatments do not allow satisfactory conclusions to be drawn with regard to the effect of this cation upon per cent dry weight. The general statement holds true that the treatments which produced

plants of high average green weight also produced plant tissue of a low percentage of dry weight, with the exception of those treatments high in  $Mg^{++}$ , which were irregular in this respect.

The fact should be emphasized that only four of the solutions of Shive's triangle have been dealt with in these experiments. The triangle, represents 36 different solutions, any of which might be used in similar experiments. A selection of several of the solutions which customarily produce high yields, and a variation of the ionic concentrations in some regulated manner such as has been used in these experiments, may prove of considerable value in nutritional work.

#### SUMMARY AND ABSTRACT

A system of physiologically balanced solutions is presented in which it is possible to vary the concentration of any or all of the individual ions and to isolate the effect of any ion or combination of ions upon plant growth, as measured by average green weight per plant.

The series of solutions was used in the growth of a strain of hybrid corn, and the growth of the plants was used as a means of comparison of the effects of the ions.

A statistical analysis was employed with the data obtained, and the following conclusions were drawn from a consideration of the indexes of part correlation and of the net regression curves:

No correlation was observed between the amounts of  $SO_4^{--}$  or  $PO_4^{--}$  in the substrate and the growth of the plants within the limits of the concentrations employed.

Increasingly high concentrations of  $NO_3^-$  in the substrate, between the limits of one and eight out of a total of ten relative parts of the anion concentration, gave the most marked correlations with increases in growth.

Increasingly high concentrations of both  $K^+$  and  $Ca^{++}$ , between the same concentration limits, gave marked increases in growth.

Increasingly high concentrations of  $Mg^{++}$ , between the same limits, resulted in decreased growth, producing symptoms of toxicity in the high concentration utilized. The net regression curve had a negative slope within the limits of the variation of the  $Mg^{++}$  supply in the substrate.

The importance of the relative concentrations of the cations is entirely dependent upon the amount of  $NO_3^-$  present in the substrate. At low concentrations of  $NO_3^-$  little importance can be attached to the relative proportions of the three cations. At higher concentrations of  $NO_3^-$ , the necessity for higher relative proportions of  $K^+$  and  $Ca^{++}$  and for lower relative proportions of  $Mg^{++}$  in the substrate for maximum response to the  $NO_3^-$ , is brought out. With high concentrations of both  $Mg^{++}$  and  $NO_3^-$ , the toxicity of the  $Mg^{++}$  is particularly noticeable in the manner in which it inhibits the growth of the plants.

None of the three cation ratios,  $Ca/Mg$ ,  $K/Mg$ , or  $K/Ca$ , show any significance in these experiments.

The dry weight data showed correlations between the percentage of dry



weight and relative concentrations of three of the major nutritional elements. The higher the available relative amounts of both  $\text{NO}_3^-$  and  $\text{K}^+$ , the lower the percentage of dry weight of the tissues produced. With increasing  $\text{Ca}^{++}$  concentration in the medium, there was a higher percentage of dry material in the tissues.

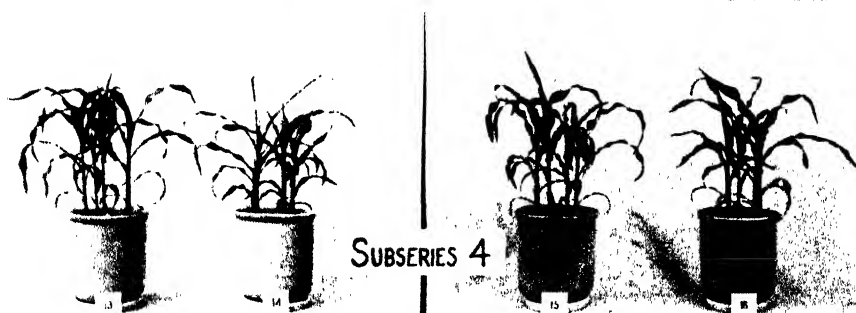
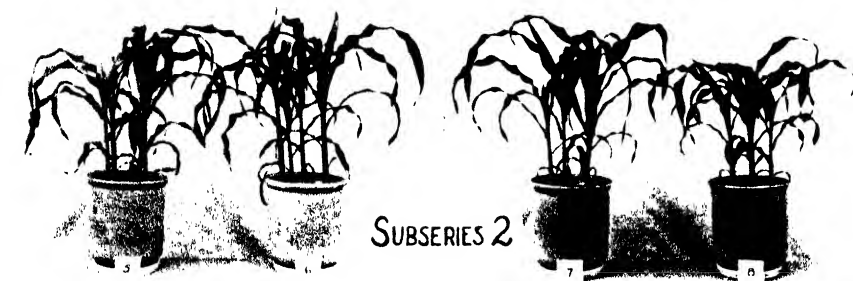
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#### PLATE 1

PLANTS FROM THE VARIOUS TREATMENTS ARRANGED IN SUBSERIES FOR COMPARISON OF ANION EFFECTS UPON GROWTH

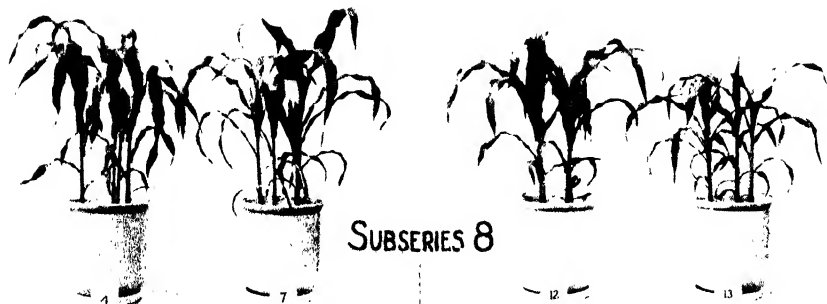
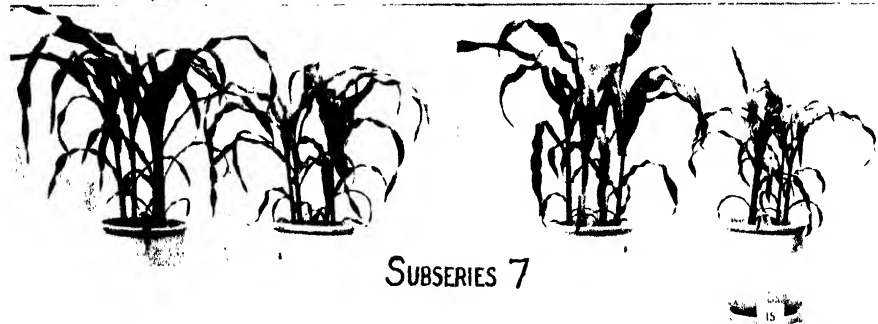
The numbers at the base of the cultures correspond to the treatment numbers given in table 3.



## PLATE 2

PLANTS FROM THE VARIOUS TREATMENTS ARRANGED IN SUBSERIES FOR COMPARISON OF  
CATION EFFECT UPON GROWTH

The numbers at the base of the cultures correspond to the treatment numbers given in table 4.





## AUTHOR INDEX

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